PTO/PCT Rec'd 1 3 FEB 2002

MELANOCORTIN METALLOPEPTIDE CONSTRUCTS,

COMBINATORIAL LIBRARIES AND APPLICATIONS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of the filing of U.S. Provisional Patent Application Serial No. 60/148,994, entitled *Melanocortin Receptor-Specific Metallopeptide Constructs, Combinatorial Libraries and Applications*, filed on August 13, 1999, and the specifications thereof is incorporated herein by reference.

This application is related to International Patent Application Serial No. PCT/US99/29743, entitled *Metallopeptide Combinatorial Libraries and Applications*, filed December 14, 1999, U.S. Pat nt No. 6,027,711, entitled *Structurally Determined Metallo-Constructs and Applications*, issued February 22, 2000, and U.S. Patent No. 5,891,418, entitled *Peptide - Metal Ion Pharmaceutical Constructs and Applications*, issued April 6, 1999, the teachings of all of which are incorporated herein by reference as if set forth in full.

GOVERNMENT RIGHTS

The U.S. Government has a paid-up license in this invention and the right in limited circumstances to require the patent owner to license others on reasonable terms as provided for by the terms of Grant No. R43 CA83417 awarded by the National Cancer Institute of the National Institutes of Health of the Department of Health and Human Services and Grant No. R43 DK55470 awarded by the National Institute of Diabetes and Digestive and Kidney Diseases of the National Institutes of Health of the Department of Health and Human Services.

BACKGROUND OF THE INVENTION

Field of the Invention (Technical Field):

The present invention relates to metallopeptides, metal ion-complexed peptidomimetics, and metallo-constructs, including metallopeptide combinatorial libraries, metal ion-complexed peptidomim tic and peptide-like combinatorial libraries and metallo-construct combinatorial libraries, specific for melanocortin receptors, including methods for the use and making of the same. The invention also relates to methods for synthesizing and assembling such libraries, and methods for identification and characterization of library constituents which are capable of binding a melanocortin receptor of interest, or mediating a melanocortin receptor-related biological activity of interest.

Background Art:

Not that the following discussion refers to a number of publications by author(s) and year of publication, and that du to recent publication dates certain publications ar not to be considered as prior art vis-a-vis the present invention. Discussion of such publications h rein is giv n for more

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complete background and is not to be construed as an admission that such publications are prior art for pat ntability d termination purposes.

Melanocortin Receptors. A family of melanocortin receptor types and subtypes have been identified, including melanocortin-1 receptors (MC1-R) expressed on normal human melanocytes and melanoma cells, melanocortin-2 receptors (MC2-R) for ACTH (adrenocorticotropin) expressed in cells of the adrenal gland, melanocortin-3 and melanocortin-4 receptors (MC3-R and MC4-R) expressed primarily in cells in the hypothalamus, mid-brain and brainstem, and melanocortin-5 receptors (MC5-R), expressed in a wide distribution of peripheral tissues.

Peptides specific for melanocortin receptors have been reported to have a wide variety of biological activities, including effects upon pigmentation and steroidogenesis, known to be mediated by MSH (melanocyte stimulating hormone) and ACTH receptors. Several studies have documented the presence of melanotropin receptors on primary human melanoma cells (Tatro JB, Atkins M, Mier JW, et al. Melanotropin receptors demonstrated in situ in human melanoma. *J Clin Invest*, 85:1825-1832, 1990). Melanotropin receptors have been reported as markers for melanotic and amelanotic human melanoma tumors (Sharma SD, Granberry ME, Jiang J, et al. Multivalent melanotropic peptide and fluorescent macromolecular conjugates: new reagents for characterization of melanotropin receptors. *Bioconjug Chem* 5:591-601, 1994; Sharma SD, Jiang J, Hadley ME, et al. Melanotropic peptide-conjugated beads for microscopic visualization and characterization of melanoma melanotropin receptors. *Proc Natl Acad Sci U S A* 93(24):13715-13720, 1996). In particular, the presence of MC1-R has been demonstrated in human melanoma cells by an antibody to MC1-R (Xia Y, Skoog V, Muceniece R, et al. Polyclonal antibodies against human melanocortin MC-1 receptor: Preliminary immunohistochemical localization of melanocortin MC1 receptor to malignant melanoma cells. *European J Pharmacol* 288:277-283, 1995). MC1-R is a G protein-coupled, 7-transmembrane receptor expressed in skin-cell melanocytes and shares some degree of homology with related receptors MC2-R, MC3-R, MC4-R and MC5-R. Each of these receptors can bind various peptide analogs that contain a common melanotropic pharmacophore, His-Phe-Arg-Trp, which describes the 6-9 sequence of the alpha-melanocyte stimulating hormone (α-MSH).

Prior to molecular characterization of the MC receptors, α-MSH analogs were labeled with the radioisotope Indium-111 and used in melanoma imaging studies (Wraight EP, Bard DR, Maughan TS, et al. The use of a chelating derivative of alpha melanocyte stimulating hormone for the clinical imaging of malignant melanoma. *Brit J Radiology* 65: 112-118, 1992; Bard DR, Knight CG and Page-Thomas DP. A chelating derivative of alpha-melanocyte stimulating hormone as a potential imaging agent for malignant melanoma. *Brit J Cancer* 62:919-922, 1990; Bard DR, Knight CG, Page-Thomas DP. Targeting of a chelating derivative of a short chain analogue of alpha-melanocyte stimulating hormone to Cloudman S91 m lanomas. *Biochem Soc Trans* 18:882-883, 1990). Linear and cyclic disulfide-containing peptides have been id ntified and used for m lanoma imaging and appear to be non-selective among MC receptors (Chen J and Quinn TP. Alpha melanocyte stimulating hormone analogu s Tc-99m/Re-188 lab ling and their pharmacokinetics in malignant melanoma bearing mice. *J Nucl Med* 39: 222p, 1998; Giblin MF, Wang N, Hoffman TJ, et al. Design and characterization of alpha-melanotropin peptide

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analogs cycliz d through rh nium and t chn tium m tal coordination. *Proc Natl Acad Sci U S A* 95(22):12814-12818, 1998). In later studi s, th cyclic p ptide r port d by Giblin and coworkers was also found to localize in the brain (Wang NN, Giblin MF, Hoffman TJ, et al. In vivo charact rization of Tc-99m and Re-188 labeled cyclic melanotropin peptide analogues in a murine melanoma model. *J Nucl*
5   *Med* 39: 77p, 1998 and corresponding poster presentation at the 45th Society of Nuclear Medicine Meeting, Toronto, June 1998). It has been recently reported that the response of human melanocytes to UV radiation is mediated by α-MSH induced activation of the cAMP pathway through the MC1-R (Im S, Moro O, Peng F, et al. Activation of the cyclic AMP pathway by alpha-melanotropin mediates the response of human melanocytes to ultraviolet B radiation. *Cancer Res* 58: 47-54, 1998).

10   MC4-R is also a G protein-coupled, 7-transmembrane receptor, but is believed to be expressed primarily in the brain. Inactivation of this receptor by gene targeting has been reported to result in mice with the maturity-onset obesity syndrome that is associated with hyperphagia, hyperinsulinemia, and hyperglycemia (Huszar D, Lynch CA, Fairchild-Huntress V, et al. Targeted disruption of the melanocortin-4 receptor results in obesity in mice. *Cell* 88:131-141, 1997). MC4-R is a molecular target
15   for therapeutic intervention in energy homeostasis.

Alpha-MSH has been described as a potent anti-inflammatory agent in all major forms of inflammation (Star RA, Rajora N, Huang J, Stock RC, Catania A, and Lipton JM: Evidence of autocrine modulation of macrophage nitric oxide synthase by alpha-melanocyte stimulating hormone. *Proc Natl Acad Sci U S A* 92:8016-8020, 1995; Getting SJ, and Perretti M: MC3-R as a novel target for
20   antiinflammatory therapy. *Drug News and Perspectives* 13:19-27, 2000). Implication of both MC1-R and MC3-R receptors in anti-inflammatory processes has been stressed. In particular, the activation of these MC receptors by melanocortin receptor agonists has been reported to inhibit the expression of nitric oxide synthase and subsequent nitric oxide production.

Significant work has been done in determining the structure of melanocortin receptors, including
25   both the nucleic acid sequences encoding for the receptors and the amino acid sequences constituting the receptors. See, for example, International Patent Applications No. PCT/US98/12098 and PCT/US99/16862 and U.S. Patent No. 5,994,087. A large number of ligands specific for melanocortin receptors, both agonists and antagonists, have also been developed. See, for example, International Patent Applications No. PCT/US98/03298 (iodo group-containing melanocortin receptor-specific linear
30   peptide), PCT/GB99/01388 (MC1-R specific linear peptides), PCT/GB99/01195 (MC3-R, MC4-R and MC5-R specific cyclic peptides), PCT/US99/04111 (MC1-R specific peptide antagonists for melanoma therapy), PCT/US99/09216 (isoquinoline compounds as melanocortin receptor ligands), PCT/US99/13252 (spiropiperdine derivatives as melanocortin receptor agonists), and U.S. Patent No. 6,054,556 (cyclic lactam peptides as MC1-R, MC3-R, MC4-R and MC5-R antagonists). In addition,
35   a large number of patents teach various methods of scre ning and determining melanocortin receptor-specific compounds, as for example International Patent Applications No. PCT/US97/15565, PCT/US98/12098 and PCT/US99/16862 and U.S. Patents No. 5,932,779 and 5,994,087.

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In gen ral, compounds specific for MC1-R ar beli ved to b useful for treatm nt of melanoma, including use as radiotherapeutic or drug delivery agent, and as diagnostic imaging agents, particularly when labeled with a diagnostic radionuclide. Compounds specific for MC3-R, MC4-R or MC5-R are believed to be useful in regulation of energy homeostasis, including use as agents for attenuating food intake and body weight gain, for use in treatment of anorexia, as a weight gain aid, for treatment of obesity, and other treatment of other food intake and metabolism-related purposes. Compounds specific for MC3-R and MC4-R, among other melanocortin receptors, can be used as agents for treatment of sexual dysfunction, including male erectile dysfunction. Compounds specific for MC3-R and MC4-R, among other melanocortin receptors, can be used to regulate blood pressure, heart rate and other neurophysiologic parameters. Other melanocortin receptor peptides can be used as tanning agents, to increase melanin production, such as peptides that are MCR-1 agonists. Compounds specific for MCR-1 and MCR-3 may be useful in regulation of inflammatory processes.

There remains a significant need for ligands with high specificity for discrete melanocortin receptors, as well as ligands or compounds that are either agonists or antagonists of specific melanocortin receptors. High affinity peptide ligands of melanocortin receptors can be used to exploit varied physiological responses associated with the melanocortin receptors, either as agonists or antagonists. In addition, melanocortin receptors have an effect on the activity of various cytokines, and high affinity peptide ligands of melanocortin receptors can be used to regulate cytokine activity.

Peptide Libraries and Combinatorial Chemistry. Libraries of peptides and other small molecul s, with enormous pool of structurally diverse molecules, are well suited for pharmaceutical lead generation and lead optimization. Libraries of a variety of molecular species have been described in literature and screened for drug discovery, including peptides, peptoids, peptidomimetics, oligonucleotides, benzodiazepines, and other libraries of small organic molecules. Various approaches have been used to construct libraries of structurally diverse chemical compounds, include chemical synthesis and genetic engineering methods. Chemically synthesized libraries have been synthesized by general solution chemical means and by solid-phase methods. The prior art on designing, synthesizing, screening, and evaluation of peptide-based libraries has been reviewed in numerous articles.

Spatially Addressable Parallel Synthesis of Solid Phase Bound Libraries. Various strategies for chemical construction of a library of peptides or other small molecules are well established. One strategy involves spatially separate synthesis of compounds in parallel on solid phase or on a solid surface in a predetermined fashion so that the location of one compound or a subset of compounds on the solid surface is known. Other methods, such as light-directed spatially addressable parallel chemical synthesis techniques, based upon use of photolithographic techniques in peptide synthesis on a solid surface, such as a borosilicate glass microscope slide, provide libraries containing more than 100,000 spatially separat d compounds. How ver, synthesis of libraries that are structurally more diverse than simple peptides requires the development of orthogonal photolabile protecting groups that can be cleaved at different wavelengths of light. In addition, the solid surface bearing these libraries also has been reported to cause a pronounced effect on binding affinities in library screening assays.

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Pooling and Split Synthesis Strategies. Large librari s of compounds can b ass mbled by a
pooling strategy that employs equimolar mixtures of reactants in each synth tic st p or by adjusting the
relative conc ntration of various r actants in the mixture according to th ir reactivities in ach of the
coupling reactions. In one approach equimolar mixtures of compounds are obtained by splitting the

5   resin in equal portions, each of which is separately reacted with each of the various monomeric
reagents. The resin is mixed, processed for the next coupling, and again split into equal portions for
separate reaction with individual reagents. The process is repeated as required to obtain a library of
desired oligomeric length and size. This approach is the basis of the "one-bead one-peptide" strategy
which employs amino acid sequencing to ascertain the primary structure of the peptide on a hit bead in a

10   bioassay. Automated systems have been developed for carrying out split synthesis of these libraries
with rather more efficiency. A common artifact occasionally seen with all these resin bound libraries is
altered target-specific affinity by some solid phase bound compounds in bioassays, which can result in
totally misleading results.

Another strategy involves construction of soluble libraries. This strategy involves a deconvolution

15   process of iterative re-synthesis and bioassaying until all the initially randomized amino acid positions
are defined. Several modifications to this strategy have been developed, including co-synthesis of two
libraries containing orthogonal pools, which eliminates the need of iterative re-synthesis and evaluation.
A major limitation of the soluble library approach is its applicability to high affinity systems. The
abundance of each compound in solution can be influenced by the total number of compounds in a

20   library that can affect the biological activity. For this reason, a highly active compound in any pool may
not in fact be the most potent molecule. Lack of reasonable solubilities of certain members in a library
may further influence this phenomenon.

Among the various classes of libraries of small molecules, peptide libraries remain the most
versatile because of the structural diversity offered by the use of naturally occurring amino acids,

25   incorporation of a variety of "designer" amino acids, and the high efficiency and ease with which peptide
synthesis can be accomplished. In addition, another level of structural diversity in peptide-based
libraries has been added by post-synthesis modification of the libraries. These modifications include
permethylation, acylation, functionalization of the side chain functionality, and reductive amination of the
N-terminus.

30   SUMMARY OF THE INVENTION (DISCLOSURE OF THE INVENTION)

In one preferred embodiment, the invention provides a construct comprising a metal ion-binding
domain comprising two or more linked residues forming an N_3S_1 ligand available for complexing with a
metal ion, wherein the construct is conformationally constrained in a structure specific for one or more
melanocortin receptors upon complexing the metal ion-binding domain with a metal ion.

35   In another pr f rred embodiment, the invention provides a manufactur d peptide and
pharmaceutically acceptable salts th reof comprising a metal ion-binding domain comprising two or
mor contiguous amino acids and a determined biological-function domain specific for one or more
m lanocortin receptors, wher in at least a portion of said biological-function domain is co-extensive with

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at least a portion of th   metal ion-binding domain, and wherein said biological-function domain is conformationally constrain d upon complexing the m tal ion-binding domain with a metal ion.

In another preferred embodiment, the invention provides a combinatorial library targeted to melanocortin receptors of different sequence peptide members synthesized on solid phase, where each

5   constituent library member comprises:

(a)   a peptide sequence of three or more amino acid residues bound to solid phase characterized by (i) a sequence of two or more amino acid residues forming a metal ion-binding domain and including at least one amino acid residue containing at least one S wherein the said S is protected by an orthogonal S-protecting group, (ii) a sequence of one or more amino acid residues at the N- or C-

10   terminus of the metal ion-binding domain, or at both the N- and C-terminus of the metal ion-binding domain, and (iii) a cleavable bond attaching the peptide sequence to solid phase; and

(b)   a unique selection or sequence of amino acid residues in the peptide sequence of at least one of the constituent members of the library;

wherein the orthogonal S-protecting group may be removed without cleaving the peptide

15   sequence from the solid phase.

In another preferred embodiment, the invention provides a combinatorial library targeted to melanocortin receptors of different sequence peptidomimetic members synthesized on solid phase, where each constituent library member comprises:

(a)   a peptidomimetic sequence of a combination of three or more amino acid residues and

20   mimics of amino acid residues bound to solid phase characterized by (i) a sequence of two or more amino acid residues, mimics of amino acid residues or combinations thereof forming a metal ion-binding domain and including at least one amino acid residue or mimic of an amino acid residue containing at least one S wherein the said S is protected by an orthogonal S-protecting group, (ii) a sequence of one or more amino acid residues, mimics of amino acid residues or combinations thereof at the N- or C-

25   terminus of the metal ion-binding domain, or at both the N- and C-terminus of the metal ion-binding domain, and (iii) a cleavable bond attaching the peptidomimetic sequence to solid phase; and

(b)   a unique selection or sequence of amino acid residues, mimics of amino acid residues or combinations thereof in the peptidomimetic sequence of at least one of the constituent members of the library;

30   wherein the orthogonal S-protecting group may be removed without cleaving the peptidomimetic sequence from the solid phase.

In another preferred embodiment, the invention provides a combinatorial library targeted to melanocortin receptors of different s quence peptide or peptidomimetic members synthesized in solution, wher   each constituent library member comprises:

35   (a)   a peptidomim tic sequence of a combination of three or more amino acid residues and mimics of amino acid residues bound to solid phase characterized by (i) a sequence of two or more

amino acid r sidu s, mimics of amino acid residues or combinations th r of forming a metal ion-binding domain and including at l ast on amino acid residu or mimic of an amino acid r sidu containing at least one S wher in the said S is protect d by an orthogonal S-prot cting group, (ii) a sequence of one or more amino acid residues, mimics of amino acid residues or combinations thereof at the N- or C-terminus of the metal ion-binding domain, or at both the N- and C-terminus of the metal ion-binding domain; and

(b)   a unique selection or sequence of amino acid residues, mimics of amino acid residues or combinations thereof in the peptidomimetic sequence of at least one of the constituent members of the library.

The compositions of this invention include compositions of the formulas:

$$R_1 - Lll - Aaa - Bbb - Ccc - R_2,$$

$$R_1 - Bbb - Aaa - Ccc - R_2,$$

$$R_1 - Ddd - Bbb - Aaa - R_3,$$

$$R_4 - Eee - Bbb - Ccc - R_2,$$

$$R_1 - Fff - Aaa - Ggg - Ccc - R_5,$$

$$R_1 - Hhh - Aaa - Bbb - Ccc - R_5, \text{ and}$$

$$R_1 - Iii - Iii - Ccc - Jjj - Kkk - R_2,$$
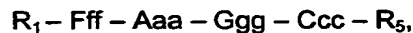
wherein

R_1 is any functionality that potentiates the intrinsic activity of the remainder of the molecule, including but not limited to providing an auxiliary or secondary receptor contact. Any of a variety of amino acids and non-peptide groups may be employed, including an amino acid chain from one to about four neutral or charged L- or D-configuration amino acid residues. If R_1 is a non-peptide group, it may be a linear or branched alkyl, aryl, alkene, alkenyl or aralkyl chain;

Aaa is an L- or D-configuration cationic amino acid with a positively charged side chain. Preferred amino acids include L-configuration Lys, Arg, Orn, Dpr or Dbu, and derivatives, analogs or homologs thereof, including both natural and synthetic amino acids. Aaa provides an N (nitrogen atom) for metal ion complexation;

Bbb is an L- or D-configuration amino acid with an aromatic side chain. Preferred amino acids include D-configuration Phe, Phe(4'Cl), Phe(3',4' Di-Cl), Phe(4'-nitro), Phe(4'-methyl), Phe(4'-Phenyl), Hphe, Pgl, Trp, 1-Nal, 2-Nal, Ser(Bzl), Lys(Z), Lys(Z-2'Br), Lys(Bz), Thr(Bzl), Cys(Bzl), or Tyr(BzlCl$_2$), and derivatives, analogs or homologs thereof. The aromatic ring in Bbb may be functionalized with halogen, alkyl or aryl groups. Bbb provides an N for metal ion compl xation;

Ccc is an amino acid that provides both an N, from the alpha amino group, and an S (sulfur atom), from a side chain group, for metal ion compl xation. Preferred amino acids include L- or D-configuration Cys, Pen and Hcys;

Lll is a D-configuration amino acid with an aromatic sid chain. Pref rred amino acids includ D-
configuration Ph , Phe(4'Cl), Ph (3',4' Di-Cl), Phe(4'-nitro), Phe(4'-methyl), Phe(4'-Phenyl),
Hphe, Pgl, Trp, 1-Nal, 2-Nal, Ser(Bzl), Lys(Z), Lys(Z-2'Br), Lys(Bz), Thr(Bzl), Cys(Bzl), or
Tyr(BzlCl$_2$), and derivatives, analogs or homologs thereof. The aromatic ring in Lll may be
5   functionalized with halogen, alkyl or aryl groups. Lll does not provide an N for metal ion
complexation;

R$_2$ is an amino acid with an aromatic side chain. Preferred amino acids include L- or D-configuration
Phe, Trp, Phe(4'Cl), Phe(3',4' Di-Cl), Phe(4'-nitro), Phe(4'-methyl), Phe(4'-Phenyl), Hphe, Pgl,
Trp, 1-Nal, 2-Nal, Ser(Bzl), Lys(Z), Lys(Z-2'Br), Lys(Bz), Thr(Bzl), Cys(Bzl) or Tyr(BzlCl$_2$), and
10   derivatives, analogs or homologs thereof, including both natural and synthetic amino acids. The
C-terminus may be free or amidated. R$_2$ may also be the corresponding des-carboxyl amino acid
of any of the foregoing. Alternatively, R$_2$ may be eliminated;

Ddd is an amino acid that provides an S, from a side chain group, for metal ion complexation. Preferr d
amino acids include L- or D-configuration Cys, Pen and Hcys;

15   R$_3$ is an amino acid with an aromatic side chain that provides an N for metal ion complexation. Preferred
amino acids include L- or D-configuration Phe, Trp, Phe(4'Cl), Phe(3',4' Di-Cl), Phe(4'-nitro),
Phe(4'-methyl), Phe(4'-Phenyl), Hphe, Pgl, Trp, 1-Nal, 2-Nal, Ser(Bzl), Lys(Z), Lys(Z-2'Br),
Lys(Bz), Thr(Bzl), Cys(Bzl) or Tyr(BzlCl$_2$), and derivatives, analogs or homologs thereof,
including both natural and synthetic amino acids. The C-terminus may be free or amidated. R$_3$
20   may also be the corresponding des-carboxyl amino acid of any of the foregoing;

R$_4$ is a functionality that provides a cationic center. Preferred amino acids include L- or D- configuration
Lys, Arg, Orn, Dpr or Dbu, and derivatives, analogs or homologs thereof, including both natural
and synthetic amino acids. The N-terminus of the amino acid may be functionalized with any of a
variety of neutral amino acid and non-peptide groups, including linear or branched alkyl, aryl,
25   alkene, alkenyl or aralkyl chains;

Eee is an uncharged L- or D-configuration amino acid that provides an N for metal ion complexation.
Preferred amino acids include Gly and L-configuration Ala, Nle, Leu, Val, Phe or Trp, and
derivatives, analogs or homologs thereof, including both natural and synthetic amino acids. In a
preferred embodiment, Eee isn an amino acid with an aliphatic side chain;

30   Fff is an L- or D-configuration aromatic amino acid. Preferred amino acids include D-configuration Phe,
Phe(4'Cl), Phe(3',4' Di-Cl), Phe(4'-nitro), Phe(4'-methyl), Phe(4'-Phenyl), Hphe, Pgl, Trp, 1-Nal,
2-Nal, Ser(Bzl), Lys(Z), Lys(Z-2'Br), Lys(Bz), Thr(Bzl), Cys(Bzl), Tyr(BzlCl$_2$), Tic, Tiq or Tca, and
derivatives, analogs or homologs thereof, including both natural and synthetic amino acids. The
aromatic ring in Fff may be substituted with halogen, alkyl or aryl groups. Fff does not provide an
35   N for metal ion complexation;

Ggg is an L- or D-configuration aromatic amino acid. Preferred amino acids include L-configuration Phe,
Phe(4'Cl), Phe(3',4' Di-Cl), Phe(4'-nitro), Ph (4'-methyl), Phe(4'-Phenyl), Hphe, Pgl, Trp, 1-Nal,

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2-Nal, Ser(Bzl), Lys(Z), Lys(Z-2'Br), Lys(Bz), Thr(Bzl), Cys(Bzl) or Tyr(BzlCl$_2$), and derivativ s, analogs or homologs th reof, including both natural and synthetic amino acids. Th aromatic ring in Ggg may be substituted with halogen, alkyl or aryl groups. Ggg provides an N for metal ion complexation;

5　　R_5 is preferably an amide, substituted amide, ester or carboxylate group. R_5 may also be and L- or D-configuration amino acid or amino acid amide, including an aromatic, aliphatic, neutral or charg d amino acid;

Hhh is an L- or D-configuration cationic amino acid with a positively charged side chain. Preferred amino acids include L-configuration Lys, Arg, Orn, Dpr or Dbu, and derivatives, analogs or

10　　homologs thereof, including both natural and synthetic amino acids. Hhh does not provide an N for metal ion complexation;

Iii is an L- or D-configuration amino acid that provides an N for metal ion complexation. Preferred amino acids includes Ala, Gly, Nle, Val. Leu, Ile, His, Lys, or Arg, and derivatives, analogs or homologs thereof, including both natural and synthetic amino acids;

15　　Jjj is an L- or D-configuration amino acid with an aromatic side chain. Preferred amino acids include D-configuration Phe, Phe(4'Cl), Phe(3',4' Di-Cl), Phe(4'-nitro), Phe(4'-methyl), Phe(4'-Phenyl), Hphe, Pgl, Trp, 1-Nal, 2-Nal, Ser(Bzl), Lys(Z), Lys(Z-2'Br), Lys(Bz), Thr(Bzl), Cys(Bzl), or Tyr(BzlCl$_2$), and derivatives, analogs or homologs thereof. The aromatic ring in Jjj may be functionalized with halogens, alkyl or aryl groups. Jjj does not provide an N for metal ion

20　　complexation; and

Kkk is an L- or D-configuration cationic amino acid with a positively charged side chain. Preferred amino acids include L-configuration Lys, Arg, Orn, Dpr or Dbu, and derivatives, analogs or homologs thereof, including both natural and synthetic amino acids. Aaa does not provide an N for metal ion complexation.

25　　　　In the compositions of this invention, the metal ion-binding domain can be complexed with a metal ion, and such compositions are included within the invention. The invention further includes compositions wherein the composition is substantially more specific for one or more melanocortin receptors when the metal ion-binding domain is complexed with a metal ion than is the composition when the metal ion-binding amino acid sequence is not complexed with a metal ion.

30　　　　The combinatorial libraries of this invention include libraries wherein the metal ion-binding domain further comprises at least one N available for binding to a metal ion upon removal of the orthogonal S-protecting group. The combinatorial libraries include compositions wherein the metal ion-binding domain comprises three residues forming an N$_3$S$_1$ ligand.

　　　　In th combinatorial libraries, the orthogonal S-protecting group is S-thio-butyl, acetamidomethyl,

35　　4-methoxytrityl, S-sulfonate or 3-nitro-2-pyridinesulfenyl. The orthogonal S-protecting group may be removed from constituent library members thereof without otherwise altering the constituent library members or any amino acid side chain protecting group therein. Inthe combinatorial libraries, structural

diversity may occurs in the metal ion-binding domain or outsid th m tal ion-binding domain.

In the combinatorial libraries, one or mor constituent library members may include at least one amino acid residue or mimic of an amino acid residue in the sequence at the N- or C-terminus of the metal ion-binding domain containing at least one S wherein the said S is protected by a non-orthogonal

5   S-protecting group, whereby the orthogonal S-protecting group may be removed without removing the non-orthogonal S-protecting group.

For combinatorial libraries limited to amino acids, the amino acid residue containing at least one S wherein the said S is protected by an orthogonal S-protecting group can be an L- or D-3-mercapto amino acid, including but not limited to L- or D-cysteine or L- or D-penicillamine. For combinatorial

10   libraries including amino acid residues and mimics of amino acid residues, the residue containing at least one S wherein the said S is protected by an orthogonal S-protecting group can be an L- or D-3-mercapto amino acid, including but not limited to L- or D-cysteine or L- or D-penicillamine; 3-mercapto phenylananine; 2-mercaptoacetic acid; 3-mercaptopropionic acid; 2-mercaptopropionic acid; 3-mercapto-3,3,-dimethyl propionic acid; 3-mercapto-3,3,-diethyl proprionic acid; 3-mercapto,3-methyl

15   propionic acid; 2-mercapto,2-methyl acetic acid; 3-cyclopentamethlene,3-mercaptopropionic acid; or 2-cyclopentamethlene,2-mercaptoacetic acid.

It is an object of this invention to devise, demonstrate and illustrate the preparation and use of highly specific conformationally restricted peptides, peptoids, related pseudopeptides, peptidomimetics and metallo-constructs formed by complexing sequences thereof to a desired metal ion so that the

20   topography of the side chains in the resulting complex is a biologically active three-dimensional structure specific for melanocortin receptors.

Another object of this invention is to provide peptide-metal ion complexes specific for melanocortin receptors and which have a higher level of stability and are less susceptible to proteolysis than either the uncomplexed peptide, or other peptides known in the art.

25   Another object of this invention is to provide peptide-metal ion complexes that are specific for different subsets of melanocortin receptors, such as specific only for MC1-R or for MC4-R.

Another object of this invention is to provide peptide-metal ion complexes which are specific for melanocortin receptors and which are agonists or antagonists.

Another object of this invention is to provide for peptide analogs which are not conformationally

30   restricted in the absence of a metal ion, whereby the uncomplexed peptide analog is either inactive or demonstrates low potency, but which is conformationally restricted on complexation with a metal ion and specific for melanocortin receptors with high potency.

Another object of this invention is to utilize metal complexation in a peptide specific for melanocortin receptors to cause specific regional conformational restrictions in th peptide so that the

35   peptide conformation at the metal binding site is conformationally fixed on metal complexation.

Another obj ct of this invention is to complex a peptid to a metal ion, wher by the resulting metallop ptid is specific for melanocortin r ceptors, and exhibits a preferred in vivo biodistribution profile, rate and mode of clearance, bioavailability and pharmacokinetics in mammals.

Another object of this invention is to provide peptide-metal ion complexes specific for melanocortin receptors utilizing stable non-radioactive metal ions, for use in therapeutic treatment of disease, including as an agent to modify energy metabolism and feeding behavior, such as for treatm nt of pathologic obesity and related conditions.

Another object of this invention is to provide peptide-metal ion complexes specific for melanocortin receptors utilizing metal ions which are radionuclides for use in diagnostic methods, including imaging and staging of melanoma tumors and melanoma tumor metastases.

Another object of this invention is to provide peptide-metal ion complexes specific for melanocortin receptors utilizing stable non-radioactive metal ions, for use as a prevention agent against ultra-violet radiation-induced DNA damages, including sunlight-induced DNA damage in the skin.

Another object of this invention is to provide peptide-metal ion complexes specific for melanocortin receptors utilizing stable non-radioactive metal ions, for use as a tanning agent, including but not limited to therapeutic use as a tanning agent.

Another object of this invention is to provide peptide-metal ion complexes specific for melanocortin receptors utilizing stable non-radioactive metal ions, for use as anti-inflammatory agents.

Another object of this invention is to complex peptides with radiometal ions, including but not limted to technetium-99m, for use in diagnostic imaging, so that the resulting peptide-metal ion complex is substantially more specific for melanocortin receptors than the uncomplexed peptide molecule, and the resulting radiolabeled species is essentially carrier-free in terms of specificity for melanocortin receptors.

Another object of this invention is to complex peptides with radiometal ions, including radioisotopes of rhenium such as rhenium-186 and rhenium-188, for use in targeted radiotherapy, such as for treatment of melanoma.

Another object of this invention is to provide peptide-metal ion complexes specific for melanocortin receptors that can transit the gut-blood barrier, without significant enzymatic or peptidase degradation, and may be adapted for oral administration.

Another object of this invention is to provide libraries of conformationally constrained peptide-metal ion complexes directed to melanocortin receptors.

Another object of this invention is to provide combinatorial peptide libraries of peptide-metal ion complexes specific for melanocortin receptors, wherein the peptides include a metal ion-binding domain, such that a specific conformational restriction is obtained upon labeling the peptides with a metal ion.

Another object of this invention is to provide combinatorial peptide libraries of peptide-metal ion complexes specific for melanocortin r ceptors, wherein the amino acids comprising the p ptides may be

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naturally occurring amino acids, isom rs and modifications of such amino acids, non-prot in amino acids, post-translationally modifi d amino acids, enzymatically modified amino acids, constructs or structures design d to mimic amino acids, and the like, so that the library includes pseudopeptides and peptidomimetics.

5   Another object of this invention is to provide metallopeptide libraries specific for one or more melanocortin receptors, wherein the metallopeptides include a metal ion-binding domain, such that a determined conformational restriction is obtained upon labeling the peptides with a metal ion, and the metallopeptides further include distinct, unique and different amino acid sequences.

           Another object of this invention is to provide both soluble and solid phase metallopeptide libraries
10   specific for one or more melanocortin receptors, wherein the metallopeptides include a metal ion-binding domain.

           Another object of this invention is to provide methods for synthesis of peptides specific for melanocortin receptors wherein the peptide contains a reactive SH group forming a part of a metal ion-binding domain, whereby the reactive SH group is protected during synthesis, and is deprotected only
15   upon complexing the peptides with a metal ion.

           Another object of this invention is to provide combinatorial metallopeptide libraries specific for melanocortin receptors wherein the peptides forming the library contain a reverse turn structure as a consequence of metal ion complexation.

           Another object of this invention is to provide a method for rapid and efficient complexation of a
20   pool of diverse peptides specific for melanocortin receptors with a metal ion, including a rhenium metal ion.

           Another object of this invention to provide libraries of conformationally constrained peptide-metal ion complexes as surrogates for reverse turn structures, such as beta turns and gamma turns commonly found in naturally occurring peptides and proteins specific for melanocortin receptors. The turns formed
25   as a consequence of metal ion complexation are more stable than the naturally occurring turn structures, which are stabilized only by weaker interactions such as van der Waals' interactions and hydrogen bonds.

           Another object of this invention is to provide combinatorial metallopeptide libraries wherein each of the peptides forming the library contain a reverse turn structure as a consequence of metal ion
30   complexation.

           Another object of this invention is to provide a method for the identification of specific metallopeptides through internal signatures resulting from use of metal ions with two or more isotopic peaks, such as through use of rhenium containing two isotopes in fixed relative abundance that differ in mass by 2 units.

35   Other objects, advantag s and novel features, and the further scope of applicability of the present invention, will be set forth in part in the detailed description to follow, tak n in conjunction with the accompanying drawings, and in part will become apparent to those skilled in the art upon

examination of the following, or may be learned by practice of this invention. Th objects and advantages of this inv ntion may be r aliz d and attain d by means of the instrumentalities and combinations particularly pointed out in the appended claims.

BRIEF DESCRIPTION OF THE DRAWINGS

5   The accompanying drawings, which are incorporated into and form a part of the specification, illustrate several embodiments of the present invention and, together with the description, serve to explain the principles of the invention. The drawings are only for the purpose of illustrating a preferred embodiment of the invention and are not to be construed as limiting the invention. In the drawings:

Fig. 1 is a molecular structure for **Template 1**.

10   Fig. 2 is a molecular structure for **Template 2**.

Fig. 3 is a molecular structure for **Template 3**.

Fig. 4 is a molecular structure for **Template 4**.

Fig. 5 is a molecular structure for **Template 5**.

Fig. 6 is a molecular structure for **Template 6**.

15   Fig. 7 is a molecular structure for **Template 7**.

Fig. 8 is a flow chart of a split pool and combination synthesis method according to **Example 2**.

Fig. 9 is a mass spectrum of a library pool of 25 metallopeptides synthesized according to **Example 2**.

Fig. 10 is a mass spectrum of a library pool of 4 metallopeptides synthesized according to

20   **Example 6**.

Figs. 11A - 11E are reversed phased HPLC profiles of a library pool of 4 metallopeptides synthesized according to **Example 6**.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

25   ## (BEST MODES FOR CARRYING OUT THE INVENTION)

Definitions. Certain terms as used throughout the specification and claims are defined as follows:

The terms "bind," "binding," "complex," and "complexing," as used throughout the specification and claims, are generally intended to cover all types of physical and chemical binding, reactions,

30   compl xing, attraction, chelating and the like.

Th "peptides" of this inv ntion can be a) naturally-occurring, b) produc d by ch mical synthesis, c) produced by recombinant DNA t chnology, d) produced by biochemical or enzymatic fragmentation of

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larg r molecul s,) produced by methods resulting from a combination of methods a through d listed abov , or f) produced by any other means for producing peptides.

By employing chemical synthesis, a preferred means of production, it is possible to introduce various amino acids which do not naturally occur along the chain, modify the N- or C-terminus, and the
5   like, thereby providing for improved stability and formulation, resistance to protease degradation, and the like.

The term "peptide" as used throughout the specification and claims is intended to include any structure comprised of two or more amino acids, including chemical modifications and derivatives of amino acids. For the most part, the peptides of this invention comprise fewer than 100 amino acids, and
10   preferably fewer than 60 amino acids, and most preferably ranging from about 2 to 20 amino acids. The amino acids forming all or a part of a peptide may be naturally occurring amino acids, stereoisomers and modifications of such amino acids, non-protein amino acids, post-translationally modified amino acids, enzymatically modified amino acids, constructs or structures designed to mimic amino acids, and the like, so that the term "peptide" includes pseudopeptides and peptidomimetics, including structures which
15   have a non-peptidic backbone. The term "peptide" also includes dimers or multimers of peptides. A "manufactured" peptide includes a peptide produced by chemical synthesis, recombinant DNA technology, biochemical or enzymatic fragmentation of larger molecules, combinations of the foregoing or, in general, made by any other method.

The "amino acids" used in this invention, and the term as used in the specification and claims,
20   include the known naturally occurring protein amino acids, which are referred to by both their common three letter abbreviation and single letter abbreviation. *See generally* Synthetic Peptides: A User's Guide, GA Grant, editor, W.H. Freeman & Co., New York, 1992, the teachings of which are incorporated herein by reference, including the text and table set forth at pages 11 through 24. As set forth above, the term "amino acid" also includes stereoisomers and modifications of naturally occurring protein amino
25   acids, non-protein amino acids, post-translationally modified amino acids, enzymatically synthesized amino acids, derivatized amino acids, constructs or structures designed to mimic amino acids, and the like. Modified and unusual amino acids are described generally in Synthetic Peptides: A User's Guide, cited above; Hruby VJ, Al-obeidi F and Kazmierski W: *Biochem J* 268:249-262, 1990; and Toniolo C: *Int J Peptide Protein Res* 35:287-300, 1990; the teachings of all of which are incorporated herein by
30   reference. In addition, the following abbreviations have the meanings giving:

Abu	-	gamma-amino butyric acid
2-Abz	-	2-amino benzoic acid
3-Abz	-	3-amino benzoic acid
4-Abz	-	4-amino benzoic acid
Achc	-	1-amino-cyclohexane-1-carboxylic acid
Acpc	-	1-amino-cyclopropane-1-carboxylic acid
12-Ado	-	12-amino dodecanoic acid
7-Ahept	-	7-amino heptanoic acid
Aic	-	2-aminoindane-2-carboxylic acid
6-Ahx	-	6-amino hexanoic acid
8-Aoc	-	8-amino octanoic acid
Arg(Tos)	-	N^G-para-tosyl-arginine
Asp(anilino)	-	beta-anilino-aspartic acid

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Asp(3-Cl-anilino)	-	beta-(3-chloro-anilino)-aspartic acid
Asp(3,5-diCl-anilino)-		beta-(3,5-dichloro anilino)-aspartic acid
D/L Atc	-	(D,L)-2-aminot tralin-2-carboxylic acid
11-Aun	-	11-amino und canoic acid
AVA	-	5-amino valeric acid
Bip	-	biphenylalanine
Bz	-	Benzoyl
Cha	-	Cyclohexylalanine
Chg	-	Cyclohexylglycine
Dip	-	3,3-Diphenylalanine
Et-	-	Ethyl
GAA	-	epsilon-guanidino acetic acid
GBzA	-	4-guanidino benzoic acid
B-Gpa	-	3-guanidino propionic acid
GVA(Cl)	-	beta-chloro-epsilon-guanidino valeric acid
Hphe	-	Homophenylalanine
Inp	-	isonipecotic acid
Lys(Z)	-	N-epsilon-benzyloxycarbonyl-lysine
Me-	-	Methyl
Nal 1	-	3-(1-naphthyl)alanine
Nal 2	-	3-(2-naphthyl)alanine
(N-Bzl)Nal 2	-	N-benzyl-3-(2-naphthyl) alanine
(N-PhEt)Nal 2	-	N(2-phenylethyl)-3-(2-naphthyl) alanine
Phg	-	Phenylglycine
pF-Phe	-	para-fluoro-phenylalanine
Phe(4-Br)	-	4-bromo-phenylalanine
Phe(4-CF$_3$)	-	4-trifluoromethyl-phenylalanine
Phe(4-Cl)	-	4-chloro-phenylalanine
Phe(2-Cl)	-	2 chloro-phenylalanine
Phe(2, 4- diCl)	-	2,4,-dichloro-phenylalanine
Phe(3,4- diCl)	-	3,4,-dichloro-phenylalanine
Phe(3,4- diF)	-	3,4,-difluoro-phenylalanine
Phe(4-I)	-	4-iodo-phenylalanine
Phe(3,4- di-OMe)	-	3,4,-dimethoxy-phenylalanine
Phe(4- Me)	-	4-methyl-phenylalanine
Phe(4-NO$_2$)	-	4-nitro-phenylalanine
Pip	-	Pipecolic acid
Qal(2')	-	beta-(2-quinolyl)-alanine
Sal	-	3-styrylalanine
TFA	-	trifluoroacetyl
Tic	-	1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid
Tle	-	tert-butylalanine
Tyr(Bzl)	-	O-benzyl-tyrosine
Tyr(BzlDiCl 2,6)	-	O-(2,6 dichloro)benzyl-tyrosine

A single amino acid, including stereoisomers and modifications of naturally occurring protein amino acids, non-protein amino acids, post-translationally modified amino acids, enzymatically synthesized amino acids, derivatized amino acids, constructs or structures designed to mimic amino acids, and the like, including all of the foregoing, is sometimes referred to herein as a "residue."

The library constructs of this invention also include a metal ion, which may be an ionic form of any element in metallic form, including but not limited to metals and metalloids. The metal ion may, but n ed not, be radioactive, paramagnetic or superparamagnetic. The metal ion can be of any oxidation stat of any metal, including oxidation states of vanadium (V), manganese (Mn), iron (F), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), gallium (Ga), arsenic (As), selenium (Se), yttrium (Y), molybdenum (Mo), technetium (Tc), ruth nium (Ru), rhodium (Rh), palladium (Pd), silver (Ag), cadmium (Cd), indium

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(In), tin (Sn), tungsten (W), rh nium (Re), osmium (Os), iridium (Ir), platinum (Pt), gold (Au), m rcury (Hg), thallium (Tl), lead (Pb), bismuth (Bi), polonium (Po), astatine (At), samarium (Sm), europium (Eu), and gadolinium (Gd). The metal ion can also be a radionuclide of any of the foregoing, including In, Au, Ag, Hg, Tc, Re, Sn, At, Y and Cu. A preferred metal ion with a tetradentate coordination sphere is Re.

5   For radiopharmaceutical applications, or applications wherein a radioisotope is desirable for screening, an alpha-, gamma- or beta-emitting radionuclide may be employed.

The coordination sphere of various common metal ions, in general, is tetradentate to hexadentate. In one embodiment according to this invention, an amino acid or amino acid mimetic sequence is included within each library member such that it contains the desired number of groups (4

10   to 6 in most cases) for complexing with the metal. The molecule is designed so that, upon complexing with a metal, it forms a mimic of a reverse turn structure about the site of metal complexation. A metal with coordination number 4, 5 or 6, and complexing respectively with an amino acid sequence forming a tetra, penta, or hexadentate ligand, will fold and constrain the ligand. The amino acid or amino acid mimetic sequence forming a ligand is defined as the metal ion-binding domain ("MBD") of the peptide or

15   peptidomimetic. A highly flexible molecule like a peptide, in other words, is folded to form a kind of reverse turn upon its complexation with a metal. This resulting turn is a highly constrained structure in the conformational sense.

The biological-binding domain ("BBD") of the peptide or peptidomimetic is defined in the specification and claims as a sequence of one or more amino acids which constitute a biologically active

20   sequence, exhibiting binding to a melanocotin-associated receptor, including MC1-R, MC2-R, MC3-R, MC4-R and MC5-R, thereby constituting the peptide as a member of a specific binding pair. The BBD also includes any sequence, which may be consecutive amino acids or mimetics (sychnological) or non-consecutive amino acids or mimetics (rhegnylogical) which forms a melanocortin-associated ligand, which ligand is capable of forming a specific interaction with its acceptor or receptor. The term

25   "receptor" is intended to include both acceptors and receptors. The receptor may be a biological receptor. The sequence or BBD may transmit a signal to the cells, tissues or other materials associated with the biological receptor after binding, but such is not required. The BBD may be either an agonist or antagonist, or a mixed agonist-antagonist. A peptide or peptidomimetic complexed to a metal ion with such a BBD constitutes a member of a "specific binding pair," which specific binding pair is made up of

30   at least two different molecules, where one molecule has an area on the surface or in a cavity which specifically binds to a particular spatial and polar organization of the other molecule. Frequently, the members of a specific binding pair are referred to as ligand and receptor or anti-ligand.

The BBD is further defined to include the portion of a construct, wherein the construct is a peptidomimetic, peptide-like, or metallo-construct molecule, which upon binding of the construct with a

35   metal ion, is biologically active, exhibiting binding to a melanocortin receptor found on cells, tissues, organs and other biological mat rials. The BBD may, in this instance, be sychnological or rhegnylogical, and generally has the attributes and functions of a BBD of a peptide. The BBD may be coextensive with all or a portion of the MBD, so that the same amino acids or other residues which constitute the MBD also constitute all or a part of the BBD. In some instances, one or amino acids of the MBD will form a

part of the BBD, and on   or more additional amino acids, which are not part of th   MBD, form the
remainder of the BBD.

Conformational constraint refers to the stability and preferred conformation of the three-
dimensional shape assumed by a peptide or other construct. Conformational constraints include local
5   constraints, involving restricting the conformational mobility of a single residue in a peptide; regional
constraints, involving restricting the conformational mobility of a group of residues, which residues may
form some secondary structural unit; and global constraints, involving the entire peptide structure. *See
generally* Synthetic Peptides: A User's Guide, cited above.

The primary structure of a peptide is its amino acid sequence. The secondary structure deals
10   with the conformation of the peptide backbone and the folding up of the segments of the peptide into
regular structures such as α-helices, β-sheets, turns and the like. Thus, the three-dimensional shape
assumed by a peptide is directly related to its secondary structure. *See generally* Synthetic Peptides: A
User's Guide, cited above, including the text, figures and tables set forth at pages 24-33, 39-41
and 58-67. A global structure refers to a peptide structure which exhibits a preference for adopting a
15   conformationally constrained three-dimensional shape.

The product resulting from the methods set forth herein can be used for both medical
applications and animal husbandry or veterinary applications. Typically, the product is used in humans,
but may also be used in other mammals. The term "patient" is intended to denote a mammalian
individual, and is so used throughout the specification and in the claims. The primary applications of this
20   invention involve human patients, but this invention may be applied to laboratory, farm, zoo, wildlife, pet,
sport or other animals. The products of this invention may optionally employ radionuclide ions, which
may be used for diagnostic imaging purposes or for radiotherapeutic purposes.

Peptide and Metallo-Construct Molecule Libraries and Combinatorial Chemistries. Using the
methods of this invention, libraries of peptides and peptidomimetics are designed wherein each
25   constituent library member includes an MBD sequence necessary for providing a coordination site for
complexation with a metal, it being understood that such sequence may differ among the constituent
members of the library. Upon complexing the MBD with a metal, a specific structure results, forming a
mimic of a reverse turn structure. The specific stereochemical features of this complex are due to the
stereochemistry of the coordination sphere of the complexing metal ion. Thus the preferred geometry of
30   the coordination sphere of the metal dictates and defines the nature and extent of conformational
restriction.

Libraries of this invention contain constituents which are either locally or globally constrained
structures. Libraries may include molecules with either local conformation restrictions or global
conformation restrictions, or some combination thereof. This aspect of the invention includes a variety of
35   methods of synthesis, screening and structural elucidation of positive hits in screening systems. The
importance of these aspects is well known to those skilled in the art and will also become evident from
the following description and examples.

In g neral, most of the metals that may prove useful in this invention have a coordination number of 4 to 6, and rarely as high as 8, which impli s that the putative MBD must be made of residues with reactiv_ groups located in a stereocompatible manner so as to establish a bond with a metal ion of giv n geometry and coordination sphere. Coordinating groups in the peptide chain include nitrogen atoms of

5   amine, amide, imidazole, or guanidino functionalities; sulfur atoms of thiols or disulfides; and oxygen atoms of hydroxy, phenolic, carbonyl, or carboxyl functionalities. In addition, the peptide chain or individual amino acids can be chemically altered to include a coordinating group, such as oxime, hydrazino, sulfhydryl, phosphate, cyano, pyridino, piperidino, or morpholino groups. For a metal with a coordination number of 4, a tetrapeptide amino acid sequence may be employed (such as Gly-Gly-Gly-

10   Gly), or a tripeptide amino acid sequence in which at least one of the amino acids has a side chain with a coordinating group can similarly be employed (such as Gly-Gly-Cys). The side chain can have a nitrogen, oxygen or sulfur-based coordination group. Thus, an amino acid sequence can provide an N_4, N_3S, N_2S_2, NS_3, N_2SO or similar ligand, yielding tetradentate coordination of a metal ion utilizing nitrogen, sulfur and oxygen atoms.

15   In another embodiment of the invention, the MBD includes one or more amino acid residues and one or more derivatized amino acids or spacer sequences, with the derivatized amino acid or spacer sequence having a nitrogen, sulfur or oxygen atom available for complexing with the various oxidation states of the metal. Examples of derivatized amino acids include amide, primary alkyl or aryl amide, 1,2,3,4-tetrahydroisoquinoline-2-carboxylic acid and its corresponding 7-hydroxy derivative, N-

20   carboxymethylated amino acids, 2'-mercapto-Trp, N^β-(2 mercaptoethane)-α,β-diaminopropionic acid and similar higher homologs of other homologous amino acids, N^β-(2 aminoethane)-α,β-diaminopropionic acid and similar higher homologs of other homologous amino acids, N^β-(picolinoyl)-α,β-diaminopropionic acid and similar higher homologs of other homologous amino acids, β-(picolylamide)-Asp and similar homologs of other homologous amino acids, N^β-(2-amino-benzoyl)-α,β-diaminopropionic acid and similar

25   higher homologs of other homologous amino acids, β-(2-amidomethylpyridine)-Asp and similar homologs of other homologous amino acids, N-benzyloxycarbonyl amino acid, N-tert butyloxycarbonyl amino acid, N-fluorenylmethyloxycarbonyl amino acid and other similar urethane-protected amino acid derivatives, and other derivatized or synthetic amino acids relating to any of the foregoing. Examples of spacer sequences which may be employed in this invention include 2-mercaptoethylamine, succinic

30   acid, glutaric acid, 2-mercaptosuccinic acid, ethylenediamine, diethylenetriamine, triethylenetetraamine, tetraethylenepentaamine, glycol, polyethylene glycol, thioglycolic acid, mercaptopropionic acid, pyridine-2-carboxylate, picolylamine, 2-mercaptoaniline, 2-aminobenzoic acid, and 2-aminomethylpyridine. In general, any sequence which may be linked, directly or indirectly, to one or two amino acids so as to form a continuous sequence, and which has a nitrogen, sulfur or oxygen atom available for complexing

35   with the valences of the metal ion, may be employed as an element of the MBD.

S-Protected Thiol Group Compounds in Metallo-Libraries. A free thiol (SH) group is preferred for compl xation of most metal ions to the peptides and peptidomimetics of this invention, and in many cases an SH group is necessary in order to form a stable xchange-inert complex with a m tal. Peptid s and other organic molecules with free SH groups, however, are easily oxidized in air and in

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solution, and can often form a disulfide-linked dimer. If more than on fr   SH group is pres nt in a molecule, oxidation may lead to a complex polymer. Similarly, if a mixture of different peptid s or organic molecules with free SH groups are prepared, oxidation generally leads to a complex mixture of polymers of unknown composition. This is of serious concern in preparing libraries of metallopeptides or other organic molecules where one or more SH group is intended for use in metal complexation.

A variety of SH protecting groups have been employed for a variety of purposes, including radiopharmaceutical manufacture and formulation. For example, in its protected form S-Benzoyl-mercaptoacetyl-glycyl-glycyl-glycine (Bz-MAG$_3$) has been used to complex Tc-99m (^{99m}Tc) under conditions where the S-Bz group splits during ^{99m}Tc complexation. The use of S-Bz protection, how ver, is not compatible with the methods of peptide synthesis.

In order to construct metallopeptide libraries of this invention which incorporate an SH group, if mixed pool synthesis is employed the peptides must be S-protected derivatives. The SH protecting group is chosen such that (a) the synthesis of peptide derivatives with S-protecting group is compatible with methods of solution and solid phase peptide synthesis, so that the S-protecting group is stable during synthetic procedures, and (b) the S-protecting group can be deprotected in situ, without cleavage from the resin in the case of solid phase synthesis, during the metal complexation step. Many prior art methods, such as Bz-MAG$_3$, meet at most only one of the two criteria specified above (Bz-MAG$_3$ m   ts only criterion (a) above).

Use of orthogonally S-protected thiol groups permits synthesis of metallo-compounds in a singl pot. A mixture of compounds, each compound containing an orthogonal S-protected group ("OSPG"), is used for complexation with a metal ion, and it is only during metal ion complexation that the S-protected group is deprotected, and accordingly polymerization and cross-linking is avoided. This procedure thus provides homogenous libraries of metallo-compounds.

One OSPG meeting the criteria specified above, and which can be used in this invention, employs an S^{t}Bu (S-thio-butyl or S-t-butyl) group to protect the SH group. The S^{t}Bu group is stable under both the acidic and basic conditions typically employed in peptide synthesis. Further, the S^{t}Bu group may be cleaved by reduction using a suitable phosphine reagent, which reduction step may be employed immediately prior to or in conjunction with complexation of a metal ion to the peptide. Such OSPG cleavage does not cleave the peptide from the resin, or otherwise alter the structure of the peptide.

Another OSPG meeting the criteria specified above and suitable for this invention employs an S-Acm (S-acetamidomethyl) group to protect the SH group. The Acm group is also stable under the acid and base conditions usually employed during peptide synthesis. The S-Acm group may be removed by treatment of S-Acm-protected peptide or peptide resin with mercury (II) acetate or silver (I) tertrafluoroborate, which liberates the thiol peptide in its mercury or silver ion-complexed state. Free thiol-containing peptide can then be recover d by treating the mercury or silver ion and thiol complexed salts with an excess of a thiol-containing reagent, such as beta-mercaptoethanol or dithiothreitol. The resulting peptide is th n used for metal complexation. Alternatively, the mercury or silver ion and thiol

complex d peptide may b dir ctly tr ated with a metal ion compl xing reag nt to form the desired metallopeptide.

Other examples of OSPGs for m tallopeptides include 4-methoxytrityl (Mmt), 3-nitro-2-pyridinesulfenyl (Npys) and S-sulfonate (SO$_3$H). Mmt is selectively removed upon treatment with 1% TFA in dichloromethane. Npys and S-sulfonate are selectively removed by treatment with a thiol-containing reagent such as beta-mercaptoethanol or dithiothreitol or a phosphine reagent such as tributyl phosphine. The Npys group (R.G. Simmonds RG et al: *Int J Peptide Protein Res*, 43:363,1994) is compatible with Boc chemistry for peptide synthesis and the S-sulfonate (Maugras I et al: *Int J Peptide Protein Res*, 45:152, 1995) is compatible with both Fmoc and Boc chemistries. Similar OSPGs derived from homologous series of S-alkyl, or S-aryl, or S-aralkyl may also be used in this invention. A primary characterization of the OSPG is that its use results in the formation of a disulfide (S-S) bond utilizing one sulfur atom each from the thiol-containing amino acid and the protecting group. In addition, the resulting disulfide (S-S) bond is cleavable by the use of any of a variety of disulfide cleaving agents, including but not limited to phosphine- and thiol-containing reagents.

The method employing S^{t}Bu protected SH groups, or other OSPGs, may be employed for the generation of either solid phase or soluble libraries. For solid phase libraries, peptides may be synthesized by use of conventional Fmoc chemistry. In the case of conventional Fmoc chemistry, Fmoc-L-Cys-(S^{t}Bu) is coupled to an appropriate resin, via one or more intermediate amino acids, and additional amino acids are thereafter coupled to the L-Cys-(S^{t}Bu) residue. S^{t}Bu may be employed with either L- or D-Cys, and any of a variety of other amino acids, including designer or unnatural amino acids and mimics thereof, characterized by an SH group available for binding to a metal ion, including, but not limited to, 3-mercapto phenylananine and other related 3-mercapto amino acids such as 3-mercapto valine (penicillamine); 2-mercaptoacetic acid; 3-mercaptopropionic acid; 2-mercaptopropionic acid; 3-mercapto-3,3,-dimethyl propionic acid; 3-mercapto,3-methyl propionic acid; 3-mercapto-3,3,-diethyl proprionic acid; 2-mercapto,2-methyl acetic acid; 3-cyclopentamethlene,3-mercaptopropionic acid; 2-cyclopentamethlene,2-mercaptoacetic acid and related amino acids. In all these cases, S-protection can be by S^{t}Bu, S-Acm, Mmt, Npys, S-sulfonate and related groups, as described above.

Metal Ion Complexation to MBD. The complexation of metal ions to the sequences in a library, and specifically to the MBD, is achieved by mixing the sequences with the metal ion. This is conveniently done in solution, with the solution including an appropriate buffer. In one approach, the metal ion is, when mixed with the peptide or peptidomimetic constituents, already in the oxidation state most preferred for complexing to the MBD. Some metal ions are complexed in their most stable oxidation state, such as calcium (II), potassium (I), indium (III), manganese (II), copper (II), zinc (II) and other metals. In other instances, the metal must be reduced to a lower oxidation state in order to be complexed to the MBD. This is true of ferrous, ferric, stannous, stannic, technetiumoxo[V], pertechn tate, rheniumoxo[V], perrhenate and other similar metal ions. R duction may be performed prior to mixing with the sequences, simultaneously with mixing with the sequences, or subsequent to mixing with the sequ nc s. Any means of reduction of metal ions to th desired oxidation state known to the art may be employ d.

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For tetradentate coordination with a m tal ion, rhenium is a preferr d ion. Solid phas r sin bound peptide or peptidomimetic sequences may be labeled with rhenium ion by treatment with the rhenium transfer agent $ReOCl_3(PPh_3)_2$ in the presence of 1,8-Diazabicyclo[5,4,0] undec-7-ene as a

5   base. The sequences may then be cleaved from the resin. Alternatively, peptide or peptidomimetic sequences in a soluble library may similarly be labeled by treatment with the rhenium transfer agent $ReOCl_3(PPh_3)_2$ in the presence of 1,8-Diazabicyclo[5,4,0] undec-7-ene as a base. Metal complexation in the presence of 1,8-Diazabicyclo[5,4,0]undec-7-ene (DBU) as a base can conveniently be accomplished at ambient room temperature.

10   In an alternative method of metal complexation a mild base, such as sodium acetate, can be used. In this case the thiol-containing sequence, either in solution or bound to solid phase, is taken in a suitable solvent, such as DMF, NMP, MeOH, DCM or a mixture thereof, and heated to 60-70°C with the rhenium transfer agent $ReOCl_3(PPh_3)_2$ in the presence of sodium acetate for 15 minutes. Similarly, other bases such as triethylamine, ammonium hydroxide and so on, may be employed. According to

15   this invention, MeOH is a preferred choice of solvent for rhenium complexation in the case of S-deprotected peptides in solution. The solvent choice for S-deprotected peptides still attached to the solid phase is guided mainly by considerations of superior solvation (swelling) of the solid phase. DMF and NMP may be employed. Various mixtures of these solvents, also in combination with MeOH, and DCM, $CHCl_3$ and so on, may also be employed to yield optimized complexation results.

20   In one embodiment of this invention, an S^tBu protected peptide is treated in situ with rhenium transfer agent in the presence of DBU and tributylphosphine to effect S-deprotection and rhenium complexation in one pot. Alternately, complexation of rhenium to the S^tBu protected peptide in the presence of rhenium perrhenate may be accomplished by treatment with $Sn[II]Cl_2$. This reagent effects S-deprotection as well as conversion of ReO_4 state to ReO state in situ to cause complexation of the

25   rhenium to the S-deprotected peptide. A preferred procedure in this invention is the use of S^tBu protected peptide with S-deprotection by treatment with tributylphosphine, and metal complexation of the resulting peptide utilizing $ReOCl_3(PPh_3)_2$ in the presence of DBU at room temperature.

In the libraries of this invention, the MBD forms a reverse turn structure upon complexation with a metal ion, with the library constructed such that side chains of amino acids within the MBD are varied,

30   and similarly amino acids not forming a part of the MBD are also varied. Various compounds in a library of metallopeptides can be obtained by varying the sequence of amino acids in a set of peptides that are all optimized to form a complex of nearly similar geometry when coordinated with a metal ion. This optimization can be obtained, for example, by appropriate positioning of amino acids having high affinity to complex a metal ion. Examples of naturally occurring amino acids with high affinity for metal

35   complexation include Cys and His. A library of such peptides, therefore, would have at l ast one of th se amino acids that is suitably placed in the sequence, with this amino acid being common to all the molecules in the library, with this amino acid thus non-randomized.

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A conceptual, generaliz d view of a solid phas library of metallopeptid s that is constructed using local conformational r striction is:

$$
\begin{array}{c}
A_2 \quad\quad O \\
\underset{A_1}{\overset{\displaystyle O \;\; N \quad N}{\quad\quad M \quad\quad}} \;\; CO\text{--}\textit{Peptide Chain--Resin} \\
N \quad S \\
CO \\
\textit{Peptide Chain}
\end{array}
$$
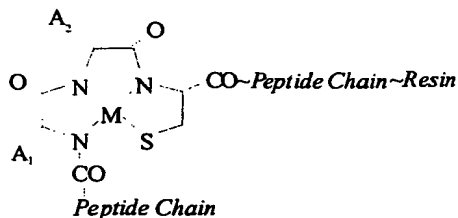
where M is a metal ion, A_1 and A_2 are amino acid side chains forming parts of the reverse turn structur which is the BBD, and "Peptide Chain" denotes one or more amino acids. A similar library can also be constructed in which the components are soluble, and thus not bound to a resin.

Another embodiment of this invention provides for construction of a library with global conformational restriction. In this embodiment, the MBD can be held constant, and a randomized or selected series of sequences of amino acids or mimetics varied to form the library. This type of library encompasses metallopeptides in which a MBD is an isosteric replacement for a disulfide, lactam, lactone, thioether or thioester moiety in cyclic peptides. In these constructs a set MBD is introduced between two pre-selected ends of a linear peptide or peptidomimetic that contains the randomized or selected series of sequences of amino acids or mimetics under investigation. The general structure of a metallopeptide library of this type is:

$$
\begin{array}{c}
A_2 \quad\quad O \\
\underline{\quad\quad\quad} \\
O \quad N \quad\; N \quad CO \\
M \\
A_1 \quad N \quad\; S \\
CO \\
\end{array}
$$
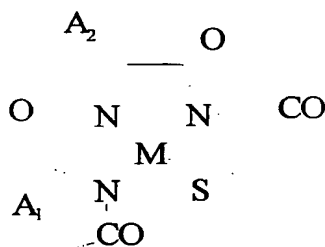
Peptide Chain (Containing Biological Function Domain)~ Resin

where M is a metal ion and A_1 and A_2 are structural elements that may provide additional stability to metal complexation, or may modulate biological activity, such as determining the organ of clearance, or altering biodistribution patterns or pharmacokinetics. The "Peptide Chain" sequence may be randomly varied, thereby resulting in a random library, or may be directed in a predetermined fashion, based upon known characteristics of the target molecule.

One illustration of a globally-constrained metallopeptide library is a library of peptides wherein all th individual members of th library include a metal ion-binding domain and the library is direct d specifically towards a family of melanocortin receptors. The general formula of this library of peptides, b fore complexation to a metal ion, is:

$$\overset{\displaystyle \lceil \text{—X—} \rceil}{A_1\text{-}Aa_1\text{-}Aa_2\text{-}Aa_3\text{-}A_2\text{-}Resin}$$
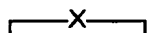
where X is a fixed MBD including a plurality of amino acids, so that all of the valences of the metal ion

5   are satisfied upon complexation of the metal ion with X, A_1 and A_2 each comprise from 0 to about 20 amino acids, and Aa_1, Aa_2 and Aa_3 each comprise one or more amino acids connected to X through an amide, thioether, thioester, ester, carbamate, or urethane bond, wherein each of Aa_1, Aa_2 and Aa_3 is varied. In this example, the MBD may include an OSPG. Other thiols in the sequence may optionally include S-protecting groups that are not orthogonal, such that the OSPG may be removed without

10   removal of other S-protecting groups in the sequence.

For solid phase libraries the peptide constructs are attached to a resin, and the resin is omitted for soluble libraries. The functional equivalent of each these peptide libraries may also be obtained through the development of a library of non-amino acid building blocks so as to result in structural mimics of these peptides. The peptide bonds may be replaced by pseudopeptide bonds, such as

15   thioamides, thioethers, substituted amines, carbanate, urethane, aliphatic moieties, and functionally similar constructs.

A peptide library is first assembled according to the sequence specification and degeneration, as described above, by well-known methods of peptide synthesis. These libraries can be synthesized as discreet, spatially addressable compounds in parallel synthesis, using split synthesis approaches, or by

20   deconvolution techniques of soluble libraries. Using similar methods, a pseudopeptide, peptidomim tic or non-peptide library can be obtained. The non-peptide libraries may also optionally incorporate one of various tagging approaches that are well known to those skilled in the art. Both solid-phase and soluble libraries can be obtained in this manner. The entire library is then reacted with an appropriate metal-complexing agent to obtain the corresponding metal-coordinated library, comprising a similar class of

25   predetermined structures. For example, to complex a peptide library with rheniumoxo metal ion, the peptide library can be treated with $Re(O)Cl_3(PPh_3)_2$ in the presence of sodium acetate. This procedure results in quantitative complexation of ReO with the peptide. In order to complex Zn, Co, Mn, Fe or Cu ions, the peptide library is treated with chloride or other suitable salts of these metal ions to yield the library of corresponding metal ions. Essentially, a variety of metal ions can be used to construct different

30   metallopeptide libraries. One limiting factor in selection of the appropriate metal ion is the relative stability of a particular metal-peptide complex, related in large part to the metal-peptide binding constant or constants. It is well known in the art that some metal-peptide constructs are stable only within specified pH or other special conditions, or are easily oxidized in air. Some peptide-metal ion complexes, such as those with ReO, are stable in pure form and can be isolated and stored under

35   normal storage conditions for a long period of time.

A metallopeptide library constructed according to this invention can be scr ened to identify one or more receptor-binding or pharmacologically-active melanocortin receptor-specific candidates by various techniques that have been reported in the prior art. Both soluble and solid phase libraries may

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b dir ctly employ d in these assays. Th s techniques include direct target binding approaches as described by Lam and cowork rs (Lam KS et al: *Nature* 354:82-84, 1991; Lam KS et al: *Nature* 360:768, 1992), deconvolution and iterativ re-synth sis approaches (Houghten RA et al: *Proc Natl Acad Sci USA* 82:5131-5135, 1985; Berg et al: *J Am Chem Soc* 111:8024-8026, 1989; Dooley CT et al: *Science*

5   266:2019-2022, 1994; Blondelle SE: *Antimicrob Agents Chemother* 38:2280-2286, 1994; Panilla C: *Biopolymers* 37:221-240, 1995), approaches using orthogonal pools of two co-synthesized libraries according to Tartar and coworkers (Deprez B et al: *J Am Chem Soc* 117: 5405-5406, 1995), positional scanning methods devised by Houghton and coworkers that eliminate iterative re-synthesis (Dooley CT et al: *Life Sci* 52:1509-1517, 1993; Pinilla C et al: *Biotechniques* 13:901-905, 1992; Pinilla C et al: *Drug*

10 *Dev Res* 33:133-145, 1992), and a combination of the positional scanning method with split synthesis methods (Erb E et al: *Proc Natl Acad Sci USA*, 91:11422-11426, 1994).

Among these techniques, the deconvolution and iterative resynthesis approach, the approach involving orthogonal pools of two co-synthesized libraries, and the positional scanning method may be directly applied to soluble metallopeptide libraries to elucidate the structure of a "hit," or peptide identified

15 as a receptor-binding or pharmacologically-active candidate in the screening process. For solid phase libraries, other than spatially addressable parallel synthesis libraries, the structure of hits can be directly determined by various strategies well known to those skilled in the art. These include direct mass spectrometric analysis of compounds covalently bound to solid phase matrix of particles by the use of matrix-assisted laser desorption/ionization (MALDI) techniques (Siuzdak G et al: *Bioorg Med Chem Lett*

20 6:979, 1996; Brown BB et al: *Molecular Diversity* 1:4-12, 1995). The technique of creating a series of partially end-capped compounds at each of the synthetic steps during library assembly also helps in unambiguous identification by mass spectrometry (Youngquist RS et al: J Am Chem Soc, 117:3900-3906, 1995; Youngquist RS et al: *Rapid Commun Mass Spectr* 8:77-81, 1994). In addition to these analytical techniques, various encoding strategies that have been devised for structure elucidation in

25 organic molecule-based libraries, including non-peptide and non-nucleotide libraries, may be utilized. Various encoding strategies, such as DNA encoding, peptide encoding, haloaromatic tag encoding, and encoding based on radiofrequency transponders, are now well known in the art and can be used directly in combination with metallopeptide libraries. These tagging strategies require the incorporation of the tags during the course of synthesis of libraries, which can be accomplished during the construction of a

30 metallopeptide libraries, since metal complexation is a final, post-synthesis step.

Structural Diversity of Melanocortin Receptor-Specific Library Members. Examples of some of the molecular templates which may be employed in this invention are shown below for tetradentate metal ion complexation. In general, these molecular templates define groups of metallopeptides of this invention which, by substitution as provided, give rise to libraries of metallopeptides for use in

35 determining melanocortin receptor-specific compounds, which may be either agonist or antagonist compounds. The templates are provided without the metal ion, it being understood that the compounds exhibit enhanced specificity for melanocortin receptors only upon metal ion complexation.

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R₁ – LII – Aaa – Bbb – C   – R₂   **Template 1**

and

R₁ – Bbb – Aaa – Ccc – R₂   **Template 2**

Where R₁ is any functionality that potentiates the intrinsic activity of the remainder of the

5   molecule, including but not limited to providing an auxiliary or secondary receptor contact. Any of a variety of amino acids and non-peptide groups may be employed, including an amino acid chain from one to about four neutral or charged L- or D-configuration amino acid residues. If R₁ is a non-peptide group, it may be a linear or branched alkyl, aryl, alkene, alkenyl or aralkyl chain.

Where Aaa is an L- or D-configuration cationic amino acid with a positively charged side chain.

10   Preferred amino acids include L-configuration Lys, Arg, Orn, Dpr or Dbu, and derivatives, analogs or homologs thereof, including both natural and synthetic amino acids. Aaa provides an N (nitrogen atom) for metal ion complexation.

Where Bbb is an L- or D-configuration amino acid with an aromatic side chain. Preferred amino acids include D-configuration Phe, Phe(4'Cl), Phe(3',4' Di-Cl), Phe(4'-nitro), Phe(4'-methyl), Phe(4'-

15   Phenyl), Hphe, Pgl, Trp, 1-Nal, 2-Nal, Ser(Bzl), Lys(Z), Lys(Z-2'Br), Lys(Bz), Thr(Bzl), Cys(Bzl), or Tyr(BzlCl₂), and derivatives, analogs or homologs thereof. The aromatic ring in Bbb may be functionalized with halogen, alkyl or aryl groups. Bbb provides an N for metal ion complexation.

Where Ccc is an amino acid that provides both an N, from the alpha amino group, and an S (sulfur atom), from a side chain group, for metal ion complexation. Preferred amino acids include L- or

20   D-configuration Cys, Pen and Hcys.

Where LII is a D-configuration amino acid with an aromatic side chain. Preferred amino acids include D-configuration Phe, Phe(4'Cl), Phe(3',4' Di-Cl), Phe(4'-nitro), Phe(4'-methyl), Phe(4'-Phenyl), Hphe, Pgl, Trp, 1-Nal, 2-Nal, Ser(Bzl), Lys(Z), Lys(Z-2'Br), Lys(Bz), Thr(Bzl), Cys(Bzl), or Tyr(BzlCl₂), and derivatives, analogs or homologs thereof. The aromatic ring in LII may be functionalized with

25   halogen, alkyl or aryl groups. LII does not provide an N for metal ion complexation.

Where R₂ is an amino acid with an aromatic side chain. Preferred amino acids include L- or D-configuration Phe, Trp, Phe(4'Cl), Phe(3',4' Di-Cl), Phe(4'-nitro), Phe(4'-methyl), Phe(4'-Phenyl), Hphe, Pgl, Trp, 1-Nal, 2-Nal, Ser(Bzl), Lys(Z), Lys(Z-2'Br), Lys(Bz), Thr(Bzl), Cys(Bzl) or Tyr(BzlCl₂), and derivatives, analogs or homologs thereof, including both natural and synthetic amino acids. The C-

30   terminus may be free or amidated. R₂ may also be the corresponding des-carboxyl amino acid of any of the foregoing. Alternatively, R₂ may be eliminated.

Fig. 1 depicts the structure of **Template 1**, and **Fig. 2** depicts the structure of **Template 2**, in both cases showing coordination with a tetradenate coordination sphere metal ion, resulting in an N₃S₁ metal ion bond.

35   R₁ – Ddd – Bbb – Aaa – R₃   T mplate 3

Where R₁, Bbb and Aaa are as described above.

Where Ddd is an amino acid that provides an S, from a side chain group, for metal ion
complexation. Preferred amino acids includ   L- or D-configuration Cys, Pen and Hcys.

Where R_3 is an amino acid with an aromatic side chain that provides an N for metal ion
complexation. Preferred amino acids include L- or D-configuration Phe, Trp, Phe(4'Cl), Phe(3',4' Di-Cl),
5   Phe(4'-nitro), Phe(4'-methyl), Phe(4'-Phenyl), Hphe, Pgl, Trp, 1-Nal, 2-Nal, Ser(Bzl), Lys(Z), Lys(Z-2'Br),
Lys(Bz), Thr(Bzl), Cys(Bzl) or Tyr(BzlCl$_2$), and derivatives, analogs or homologs thereof, including both
natural and synthetic amino acids. The C-terminus may be free or amidated. R_3 may also be the
corresponding des-carboxyl amino acid of any of the foregoing.

Fig. 3 depicts the structure of **Template 3**, showing coordination with a tetradenate coordination
10   sphere metal ion, resulting in an N_3S_1 metal ion bond.

R$_4$ – Eee – Bbb – Ccc – R$_2$ **Template 4**

Where R_2, Bbb and Ccc are as described above.

Where R_4 is a functionality that provides a cationic center. Preferred amino acids include L- or D-
configuration Lys, Arg, Orn, Dpr or Dbu, and derivatives, analogs or homologs thereof, including both
15   natural and synthetic amino acids. The N-terminus of the amino acid may be functionalized with any of
a variety of neutral amino acid and non-peptide groups, including linear or branched alkyl, aryl, alkene,
alkenyl or aralkyl chains.

Where Eee is an uncharged L- or D-configuration amino acid that provides an N for metal ion
complexation. Preferred amino acids include Gly and L-configuration Ala, Nle, Leu, Val, Phe or Trp, and
20   derivatives, analogs or homologs thereof, including both natural and synthetic amino acids. In a
preferred embodiment, Eee isn an amino acid with an aliphatic side chain.

Fig. 4 depicts the structure of **Template 4**, showing coordination with a tetradenate coordination
sphere metal ion, resulting in an N_3S_1 metal ion bond.

R$_1$ – Fff – Aaa – Ggg – Ccc – R$_5$ **Template 5**
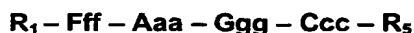
25   Where R_1, Aaa and Ccc are as described above.

Where Fff is an L- or D-configuration aromatic amino acid. Preferred amino acids include D-
configuration Phe, Phe(4'Cl), Phe(3',4' Di-Cl), Phe(4'-nitro), Phe(4'-methyl), Phe(4'-Phenyl), Hphe, Pgl,
Trp, 1-Nal, 2-Nal, Ser(Bzl), Lys(Z), Lys(Z-2'Br), Lys(Bz), Thr(Bzl), Cys(Bzl), Tyr(BzlCl$_2$), Tic, Tiq or Tca,
and derivatives, analogs or homologs thereof, including both natural and synthetic amino acids. The
30   aromatic ring in Fff may be substituted with halogen, alkyl or aryl groups. Fff does not provide an N for
metal ion complexation.

Where Ggg is an L- or D-configuration aromatic amino acid. Preferred amino acids include L-
configuration Phe, Phe(4'Cl), Phe(3',4' Di-Cl), Phe(4'-nitro), Phe(4'-methyl), Phe(4'-Phenyl), Hphe, Pgl,
Trp, 1-Nal, 2-Nal, Ser(Bzl), Lys(Z), Lys(Z-2'Br), Lys(Bz), Thr(Bzl), Cys(Bzl) or Tyr(BzlCl$_2$), and
35   derivatives, analogs or homologs thereof, including both natural and synthetic amino acids. The
aromatic ring in Ggg may be substituted with halogen, alkyl or aryl groups. Ggg provides an N for metal

ion complexation.

Where R_5 is preferably an amide, substituted amide, est r or carboxylate group. R_5 may also be and L- or D-configuration amino acid or amino acid amide, including an aromatic, aliphatic, neutral or charged amino acid.

Fig. 5 depicts the structure of **Template 5**, showing coordination with a tetradenate coordination sphere metal ion, resulting in an N_3S_1 metal ion bond.
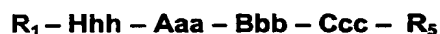
$R_1 -$ **Hhh – Aaa – Bbb – Ccc – R_5**   **Template 6**

Where R_1, Aaa, Bbb, Ccc and R_2 are as described above.

Where Hhh is an L- or D-configuration cationic amino acid with a positively charged side chain. Preferred amino acids include L-configuration Lys, Arg, Orn, Dpr or Dbu, and derivatives, analogs or homologs thereof, including both natural and synthetic amino acids. Hhh does not provide an N for metal ion complexation.

Fig. 6 depicts the structure of **Template 6**, showing coordination with a tetradenate coordination sphere metal ion, resulting in an N_3S_1 metal ion bond.

$R_1 -$ **Iii – Iii – Ccc – Jjj – Kkk – R_2**   **Template 7**

Where R_1, Ccc and R_2 are as described above.

Where Iii is an L- or D-configuration amino acid that provides an N for metal ion complexation. Preferred amino acids includes Ala, Gly, Nle, Val. Leu, Ile, His, Lys, or Arg, and derivatives, analogs or homologs thereof, including both natural and synthetic amino acids.

Where Jjj is an L- or D-configuration amino acid with an aromatic side chain. Preferred amino acids include D-configuration Phe, Phe(4'Cl), Phe(3',4' Di-Cl), Phe(4'-nitro), Phe(4'-methyl), Phe(4'-Phenyl), Hphe, Pgl, Trp, 1-Nal, 2-Nal, Ser(Bzl), Lys(Z), Lys(Z-2'Br), Lys(Bz), Thr(Bzl), Cys(Bzl), or Tyr(BzlCl$_2$), and derivatives, analogs or homologs thereof. The aromatic ring in Jjj may be functionalized with halogens, alkyl or aryl groups. Jjj does not provide an N for metal ion complexation.

Where Kkk is an L- or D-configuration cationic amino acid with a positively charged side chain. Preferred amino acids include L-configuration Lys, Arg, Orn, Dpr or Dbu, and derivatives, analogs or homologs thereof, including both natural and synthetic amino acids. Aaa does not provide an N for metal ion complexation.

Fig. 7 depicts the structure of **Template 7**, showing coordination with a tetradenate coordination sphere metal ion, resulting in an N_3S_1 metal ion bond.

The foregoing templates may be employed with tetradentate coordination sphere metal ions, such as various forms of technetium and rh nium. Corresponding templates may be constructed for use with metal ions of other coordination spher s.

Representative Peptides of this Invention. Representative peptides of this invention were made using library and synthesis methods described herein, and selected peptides w re tested using a

Where Kkk is an L- or D-configuration cationic amino acid with a positively charged sid chain. Pr ferred amino acids include L-configuration Lys, Arg, Orn, Dpr or Dbu, and d rivativ s, analogs or homologs thereof, including both natural and synth tic amino acids. Aaa does not provide an N for metal ion complexation.

5   **Fig. 7** depicts the structure of **Template 7**, showing coordination with a tetradenate coordination sphere metal ion, resulting in an N_3S_1 metal ion bond.

The foregoing templates may be employed with tetradentate coordination sphere metal ions, such as various forms of technetium and rhenium. Corresponding templates may be constructed for use with metal ions of other coordination spheres.

10   *Representative Peptides of this Invention.* Representative peptides of this invention were made using library and synthesis methods described herein, and selected peptides were tested using a binding assay. **Table 1** sets forth peptides of this invention, and the results of competitive inhibition binding assays. The peptides were synthesized using conventional peptide synthesis methods, and were complexed with rhenium using the methods described herein.

15   The competitive inhibition binding assay was conducted using membranes prepared from hMC4-R and B-16 mouse melanoma cells (containing MC1-R) using 0.4 nM ^{125}I-NDP-alpha-MSH (New England Nuclear, Boston, MA, USA) in 50 mM HEPES buffer containing 1 mM $MgCl_2$, 2 mM $CaCl_2$, and 5 mM KCl, at pH 7.2. The assay tube also contained a chosen concentration of the test peptide of this invention, complexed to a rhenium metal ion as indicated, for determining its efficacy in inhibiting the binding of ^{125}I-NDP-alpha-MSH to its receptor. Non-specific binding was measured by complete inhibition of binding of ^{125}I-NDP-alpha-MSH in the assay with the presence of 1 µM alpha-MSH. Incubation was for 90 minutes at room temperature, after which the assay mixture was filtered and the membranes washed three times with ice cold buffer. The filter was dried and counted in a gamma counter for remaining radioactivity bound to the membranes. 100% specific binding was defined as the difference in radioactivity (cpm) bound to cell membranes in the absence and presence of 1 µM alpha-MSH. The cpm obtained in presence of test compounds were normalized with respect to 100% specific binding to determine the percent inhibition of ^{125}I-NDP-alpha MSH binding. Each assay was conducted in triplicate and the actual mean valves are described in **Table 1**. Negative numbers in **Table 1** under "% Inhibition" result from experimental variance, and are indicative of no inhibition; similarly, values over 100% also result from experimental variance, and are indicative of complete inhibition.

TABLE 1					
Melanocortin Receptor Screening Results: Receptor Binding Assay				% Inhibition	
Com-pound ID	Metal ion / Linear peptid	S qu nc Structure	Conc. Cut off (µM)	MC4-R	MC1-R (B-16)
PL-808	ReO[V]	Ac-L-His-L-Trp-L-Cys-L-Trp-NH$_2$	1	0	-3
PL-809	ReO[V]	Ac-L-His-L-Hphe-L-Cys-L-Trp-NH$_2$	1	0	-4
PL-810	ReO[V]	Ac-L-His-L-2Nal-L-Cys-L-Trp- NH$_2$	1	-1	-2

		TABLE 1 Melanoc rtin R c pt r Scr ning R sults: Re pt r Binding Assay			% Inhibition	
Com-pound ID	Metal ion / Linear peptide	Sequence Structure	Conc. Cut off (μM)	MC4-R	MC1-R (B-16)	
PL-811	ReO[V]	Ac-L-His-L-Phg-L-Cys-L-Trp-NH₂	1	0	-5
PL-812	Linear	Ac-L-His-L-Phe-L-Cys-L-Trp- NH₂	10	29	54
PL-813	Linear	Ac-L-His-D-Phe-L-Cys-L-Trp- NH₂	10	8	91
PL-814	ReO[V]	Ac-L-His-L-Phe-L-Cys-L-Trp-NH₂	1	0	-4
PL-815	ReO[V]	Ac-L-His-D-Phe-L-Arg-L-Cys-L-Trp-NH₂	10	7	93
PL-816	ReO[V]	Ac-L-His-D-Phe-L-Cys-L-Trp- NH₂	10	28	70
PL-836	ReO[V]	Ac-L-His-L-Phe-D-Arg-L-Cys-L-Trp-NH₂	10	26	78
PL-837	ReO[V]	Ac-L-His-D-Phe-D-Arg-L-Cys-L-Trp-NH₂	10	0	54
PL-838	ReO[V]	Ac-L-His-L-Phe-L-Arg-L-Cys-L-Trp-NH₂	10	0	35
PL-839	ReO[V]	Ac-L-His-L-Phe-L-Trp-L-Cys-NH₂	10	9	12
PL-840	ReO[V]	Ac-L-His-L-Phe-D-Trp-L-Cys-NH₂	10	10	8
PL-841	ReO[V]	Ac-L-His-D-Phe-D-Trp-L-Cys-NH₂	10	0	19
PL-842	ReO[V]	Ac-L-His-D-Phe-L-Trp-L-Cys-NH₂	10	2	4
PL-843	ReO[V]	Ac-D-Phe-Gly-L-Cys-L-Trp-NH₂	10	1	10
PL-844	ReO[V]	Ac-L-Phe-Gly-L-Cys-L-Trp-NH₂	10	2	10
PL-845	ReO[V]	Ac-D-Phe-L-His-Gly-L-Cys-L-Trp-NH₂	10	0	5
PL-846	ReO[V]	Ac-L-Phe-D-His-Gly-L-Cys-L-Trp-NH₂	10	0	2
PL-847	ReO[V]	Ac-L-Phe-L-His-Gly-L-Cys-L-Trp-NH₂	10	6	-6
PL-848	ReO[V]	Ac-D-Phe-D-His-Gly-L-Cys-L-Trp-NH₂	10	10	19
PL-989	ReO[V]	Bz-D-Tyr-L-Nal-L-Cys-L-Phe- NH₂	10	11	35
PL-997	ReO[V]	Bz-D-Nal-L-Tyr-L-Cys-L-Phe- NH₂	10	36	6
PL-1073	ReO[V]	Ac-L-Ala-L-Tle-L-Cys-L-Trp- NH₂	1	13	-6
PL-1089	ReO[V]	Ac-L-Ala-L-pF-Phe-L-Cys-L-Trp- NH₂	10	10	0
PL-1090	ReO[V]	Ac-L-Ala-L-Tyr(3',5' di-I, 4'-Ac)-L-Cys-L-Trp-NH₂	1	10	2
PL-1091	ReO[V]	BAla-Gly-L-Cys(Bzl)-L-Cys-L-Trp- NH₂	1	13	-1
PL-1092	ReO[V]	BAla-Gly-L-Lys(TFA)-L-Cys-L-Trp- NH₂	1	4	4
PL-1093	ReO[V]	BAla-Gly-L-Phe(2,4-di Cl)-L-Cys-L-Trp- NH₂	1	2	2
PL-1094	ReO[V]	BAla-Gly-L-Phe(2-Cl)-L-Cys-L-Trp- NH₂	10	19	57
PL-1095	ReO[V]	BAla-Gly-L-Lys(Z)-L-Cys-L-Trp- NH₂	10	8	3
PL-1096	ReO[V]	BAla-Gly-L-Leu-L-Cys-L-Trp- NH₂	10	12	6
PL-1102	ReO[V]	BAla-Gly-D-Nal-2'-L-Cys-L-Trp- NH₂	10	60	9
PL-1103	ReO[V]	BAla-Gly-D-Phg-L-Cys-L-Trp- NH₂	10	12	-3
PL-1109	ReO[V]	L-Lys-L-His-D-Phe-L-Cys-L-Trp- NH₂	10	37	73
PL-1110	ReO[V]	L-Lys-L-His-L-Phe-L-Cys-L-Trp- NH₂	10	36	11
PL-1111	ReO[V]	L-His-D-Phe-L-Cys-L-Trp- NH₂	10	0	0
PL-1112	ReO[V]	L-His-L-Phe-L-Cys-L-Trp- NH₂	10	8	0
PL-1113	ReO[V]	BAla-L-His-D-Phe-L-Cys-L-Trp- NH₂	10	31	82
PL-1114	ReO[V]	BAla-L-His-L-Phe-L-Cys-L-Trp- NH₂	10	10	2
PL-1140	ReO[V]	Ac-D-Phe-Arg-L-Cys-L-Phe- NH₂	10	12	62
PL-1141	ReO[V]	Ac-D-Phe-Arg-L-Cys-L-Trp- NH₂	10	11	57
PL-1144	ReO[V]	Ac-L-Nle-L-Ala-L-His-D-Phe-L-Arg-L-Cys-L-Phe- NH₂	1	14	99
PL-1145	ReO[V]	Ac-L-Nle-L-Ala-L-His-D-Phe-L-Arg-L-Cys-L-Trp- NH₂	1	17	96
PL-1146	ReO[V]	Ac-D-Phe-Gly-L-Cys-L-Phe- NH₂	10	9	39
PL-1147	ReO[V]	Ac-D-Pip-Gly-L-Cys-L-Phe- NH₂	10	12	40
PL-1156	ReO[V]	Ac-D-Phe-L-Tle-L-Cys-L-Trp-NH₂	10	14	-19
PL-1157	ReO[V]	Ac-L-Nl -L-Arg-Gly-D-Nal 2-L-Cys-L-Trp-NH₂	10	92	53
PL-1158	ReO[V]	BAla-L-Ala-D-Nal 2-L-Cys-L-Trp-NH₂	10	62	0
PL-1159	ReO[V]	BAla-L-His-D-Nal 2-L-Cys-L-Trp-NH₂	10	63	67

TABLE 1 Melano rtin R c pt r Scre ning R sults: R c pt r Binding Assay					% Inhibition	
Com-pound ID	Metal ion / Linear peptide	Sequence Structure		Conc. Cut off (μM)	MC4-R	MC1-R (B-16)
PL-1160	ReO[V]	BAla-L-Phe-D-Nal 2-L-Cys-L-Trp-NH$_2$		10	66	1
PL-1161	ReO[V]	BAla-L-Nal 2-D-Nal 2-L-Cys-L-Trp-NH$_2$		10	64	3
PL-1162	ReO[V]	Heptanoyl-L-Arg-Gly-D-Nal 2-L-Cys-L-Trp-NH$_2$		10	79	64
PL-1163	ReO[V]	BAla-D-Ala-D-Nal 2-L-Cys-L-Trp-NH$_2$		10	51	4
PL-1164	ReO[V]	BAla-D-His-D-Nal 2-L-Cys-L-Trp-NH$_2$		10	42	2
PL-1165	ReO[V]	BAla-D-Phe-D-Nal 2-L-Cys-L-Trp-NH$_2$		10	43	7
PL-1166	ReO[V]	BAla-D-Nal 2-D-Nal 2-L-Cys-L-Trp-NH$_2$		10	44	5
PL-1167	ReO[V]	Abu-Gly-D-Nal 2-L-Cys-L-Trp-NH$_2$		10	31	-1
PL-1168	ReO[V]	6-AHX-Gly-D-Nal 2-L-Cys-L-Trp- NH$_2$		10	41	-2
PL-1169	ReO[V]	8-Aoc-Gly-D-Nal 2-L-Cys-L-Trp- NH$_2$		10	41	-3
PL-1170	ReO[V]	11-Aun-Gly-D-Nal 2-L-Cys-L-Trp- NH$_2$		10	27	9
PL-1171	ReO[V]	12-Ado-Gly-D-Nal 2-L-Cys-L-Trp- NH$_2$		10	29	-5
PL-1172	ReO[V]	Ac-L-Arg-Gly-D-Nal 2-L-Cys-L-Trp- NH$_2$		10	58	-7
PL-1173	ReO[V]	Ac-L-Lys-Gly-D-Nal 2-L-Cys-L-Trp- NH$_2$		10	41	-2
PL-1174	ReO[V]	Ac-L-Orn-Gly-D-Nal 2-L-Cys-L-Trp- NH$_2$		10	53	-2
PL-1175	ReO[V]	Ac-L-Nle-L-Arg-Gly-D-Nal 2-L-Cys-L-Trp- NH$_2$		10	96	88
PL-1176	ReO[V]	7-Ahept-Gly-D-Nal 2-L-Cys-L-Trp- NH$_2$		10	40	-8
PL-1177	ReO[V]	B-Gpa-Gly-D-Nal 2-L-Cys-L-Trp-NH$_2$		10	48	-6
PL-1178	ReO[V]	Ac-L-Nle-L-Ala-L-His-D-HPhe-L-Arg-L-Cys-L-Trp- NH$_2$		1	4	51
PL-1179	ReO[V]	Ac-L-Nle-L-Ala-L-His-D-His-L-Arg-L-Cys-L-Trp-NH$_2$		1	21	-5
PL-1180	ReO[V]	Ac-L-Nle-L-Ala-L-His-D-Trp-L-Arg-L-Cys-L-Trp-NH$_2$		1	24	86
PL-1181	ReO[V]	Ac-L-Nle-L-Ala-L-His-D-Tyr-L-Arg-L-Cys-L-Trp-NH$_2$		1	27	86
PL-1183	ReO[V]	Ac-L-Nle-L-Ala-L-Arg-D-Nal 2-L-Arg-L-Cys-L-Trp- NH$_2$		1.	82	96
PL-1184	ReO[V]	Ac-L-Nle-L-Ala-L-Tyr-D-Nal 2-L-Arg-L-Cys-L-Trp- NH$_2$		1	58	36
PL-1185	ReO[V]	Ac-L-Nle-L-Ala-L-Phe-D-Nal 2-L-Arg-L-Cys-L-Trp- NH$_2$		1	35	52
PL-1186	ReO[V]	Ac-L-Nle-L-Ala-L-Trp-D-Nal 2-L-Arg-L-Cys-L-Trp-NH$_2$		1	35	17
PL-1187	ReO[V]	Ac-L-Ala-L-His-D-Nal 2-L-Arg-L-Cys-L-Trp-NH$_2$		1	48	58
PL-1188	ReO[V]	Ac-L-Nle-L-Ala-D-Trp-D-Nal 2-L-Arg-L-Cys-L-Trp- NH$_2$		1	38	-12
PL-1189	ReO[V]	Ac-L-Nle-L-Ala-D-Phe-D-Nal 2-L-Arg-L-Cys-L-Trp- NH$_2$		1	36	-15
PL-1190	ReO[V]	Ac-L-Nle-L-Ala-D-His-D-Nal 2-L-Arg-L-Cys-L-Trp- NH$_2$		1	31	17
PL-1191	ReO[V]	Ac-L-Nle-L-Ala-D-Arg-D-Nal 2-L-Arg-L-Cys-L-Trp-NH$_2$		1	41	38
PL-1192	ReO[V]	Ac-L-Nle-L-Ala-D-Tyr-D-Nal 2-L-Arg-L-Cys-L-Tyr- NH$_2$		1	26	-5
PL-1193	ReO[V]	Ac-L-Glu-L-Ala-D-Nal 2-L-Arg-L-Cys-L-Trp-NH$_2$		1	29	51
PL-1194	ReO[V]	HOOC-(CH$_2$)$_3$-CO-L-His-D-Nal 2-L-Arg-L-Cys-L-Trp-NH$_2$		1	-3	23
PL-1195	R O[V]	Ac-L-Ala-L-Glu-L-His-D-Nal 2-L-Arg-L-Cys-L-Trp-NH$_2$		1	9	45

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		TABLE 1		% Inhibition	
		M lano rtin R cept r Scr ning Results: R ceptor Binding Assay			
Com-pound ID	Metal ion / Linear peptide	Sequence Structure	Conc. Cut off (μM)	MC4-R	MC1-R (B-16)
PL-1196	ReO[V]	Ac-L-Nle-L-Glu-L-His-D-Nal 2-L-Arg-L-Cys-L-Trp-NH$_2$	1	31	89
PL-1197	ReO[V]	Ac-L-Glu-L-His-D-Nal 2-L-Arg-L-Cys-L-Trp-NH$_2$	1	9	44
PL-1198	ReO[V]	Ac-L-Glu-L-His-D-Nal 2-L-Cys-L-Arg-NH$_2$	1	4	-5
PL-1199	ReO[V]	Ac-L-Glu-L-His-L-Cys-D-Nal 2-L-Arg-NH$_2$	1	1	-11
PL-1200	ReO[V]	Ac-L-Glu-L-His-D-Nal 2-L-Cys-L-Arg-L-Trp-NH$_2$	1	10	8
PL-1201	ReO[V]	Ac-L-Glu-L-His-L-Cys-D-Nal 2-L-Arg-L-Trp-NH$_2$	1	65	-7
PL-1202	ReO[V]	Ac-L-Nle-L-Ala-D-Trp-D-Phe-L-Arg-L-Cys-L-Trp-NH$_2$	1	17	10
PL-1203	ReO[V]	Ac-L-Nle-L-Ala-D-Nal 2-D-Nal 2-L-Arg-L-Cys-L-Trp-NH$_2$	1	35	4
PL-1204	ReO[V]	Ac-L-Nle-L-Ala-D-Nal 1-D-Nal 2-L-Arg-L-Cys-L-Trp-NH$_2$	1	32	0
PL-1205	ReO[V]	Ac-L-Nle-L-Ala-Gly-D-Nal 2-L-Arg-L-Cys-L-Trp-NH$_2$	1	54	60
PL-1206	ReO[V]	Heptanoyl-D-Trp-D-Nal 2-L-Arg-L-Cys-L-Trp-NH$_2$	1	17	0
PL-1207	ReO[V]	Ac-L-Ala-L-His-D-Nal 2-L-Cys-D-Nal 2-L-Arg-L-Trp-NH$_2$	1	84	26
PL-1209	ReO[V]	Ac-L-Nal 2-L-His-D-Nal 2-L-Cys-D-Nal 2-L-Arg-L-Trp-NH$_2$	1	77	1
PL-1210	ReO[V]	Ac-D-Nal 2-L-His-D-Nal 2-L-Cys-D-Nal 2-L-Arg-L-Trp-NH$_2$	1	71	0
PL-1211	ReO[V]	Heptanoyl-L-Glu-L-His-D-Nal 2-L-Cys-D-Nal 2-L-Arg-L-Trp-NH$_2$	1	67	26
PL-1212	ReO[V]	Ac-L-Glu-D-Trp-D-Nal 2-L-Cys-D-Nal 2-L-Arg-L-Trp-NH$_2$	1	80	0
PL-1213	ReO[V]	Ac-D-Trp-D-Arg-L-Nal 2-D-Nal 2-L-Cys-D-Nal 2-L-Arg-L-Trp-NH$_2$	1	94	34
PL-1214	ReO[V]	Heptanoyl-L-Arg-Gly-D-Nal 2-D-Nal 2-L-Cys-D-Nal 2-L-Arg-L-Trp-NH$_2$	1	78	47
PL-1215	ReO[V]	Gly-Gly-D-Nal 2-L-Cys-L-Trp- NH$_2$	10	31	20
PL-1216	ReO[V]	L-Lys-Gly-D-Nal 2-L-Cys-L-Trp- NH$_2$	10	47	0
PL-1217	ReO[V]	L-Lys(Z)-Gly-D-Nal 2-L-Cys-L-Trp- NH$_2$	10	52	24
PL-1218	ReO[V]	BAla-L-Arg(Tos)-D-Nal 2-L-Cys-L-Trp- NH$_2$	10	57	1
PL-1220	ReO[V]	BAla-L-Tle-D-Nal 2-L-Cys-L-Trp- NH$_2$	10	69	30
PL-1221	ReO[V]	BAla-L-Tyr(BzlDiCl 2,6)D-Nal 2-L-Cys-L-Trp-NH$_2$	10	35	37
PL-1222	ReO[V]	BAla-Gly-D-Phe-L-CysL-Trp- NH$_2$	10	27	9
PL-1223	ReO[V]	Ac-L-Nle-L-Arg-D-Phe-D-Nal 2-L-Cys-L-Trp-NH$_2$	10	64	79
PL-1225	ReO[V]	GBzA-Gly-D-Nal 2-L-Cys-L-Trp- NH$_2$	10	12	0
PL-1226	ReO[V]	AVA-Gly-D-Nal 2-L-Cys-L-Trp- NH$_2$	10	19	0
PL-1227	ReO[V]	2-Abz-Gly-D-Nal 2-L-Cys-L-Trp- NH$_2$	10	5	0
PL-1228	ReO[V]	BAla-L-His-Gly-D-Nal 2-L-Cys-L-Trp- NH$_2$	10	11	64
PL-1229	R O[V]	BAla-L-Nle-Gly-D-Nal 2-L-Cys-L-Trp-NH$_2$	10	32	21
PL-1230	ReO[V]	GAA-Gly-D-Nal 2-L-Cys-L-Trp- NH$_2$	10	32	20
PL-1231	ReO[V]	GVA(Cl)Gly-D-Nal 2-L-Cys-L-Trp- NH$_2$	10	32	28
PL-1232	ReO[V]	Ac-L-Nle-L-Arg-L-Ala-D-Nal 2-L-Cys-L-Trp-NH$_2$	1	90	49
PL-1233	ReO[V]	BAla-L-Arg-D-Nal 2-L-Cys-L-Trp- NH$_2$	10	94	100
PL-1234	R O[V]	BAla-D-Arg-D-Nal 2-L-Cys-L-Trp-NH$_2$	10	83	84
PL-1235	ReO[V]	Heptanoyl-L-Arg-D-Nal 2-L-Cys-L-Trp-NH$_2$	10	96	100

TABLE 1 Melanocortin R ceptor Scr ening Results: Receptor Binding Assay				% Inhibition	
Com- pound ID	Metal ion / Linear peptide	Sequence Structure	Conc. Cut off (µM)	MC4-R	MC1-R (B-16)
PL-1236	ReO[V]	Heptanoyl-D-Arg-D-Nal 2-L-Cys-L-Trp-NH$_2$	10	54	70
PL-1237	ReO[V]	BAla-Gly-D-Lys(Z)-L-Cys-L-Trp-NH$_2$	10	26	86
PL-1238	ReO[V]	BAla-Gly-D-Tyr(Bzl)-L-Cys-L-Trp-NH$_2$	10	40	40
PL-1239	ReO[V]	BAla-Gly-D-Phe(DiF 3,4)-L-Cys-L-Trp-NH$_2$	10	20	18
PL-1240	ReO[V]	BAla-Gly-D-Val-L-Cys-L-Trp-NH$_2$	10	13	0
PL-1241	ReO[V]	BAla-Gly-D-Nal 1-L-Cys-L-Trp-NH$_2$	10	34	23
PL-1242	ReO[V]	D-Nal 2-Gly-L-Arg-L-Cys-L-Trp-NH$_2$	10	61	80
PL-1243	ReO[V]	D-Nal 2-Gly-D-Arg-L-Cys-L-Trp-NH$_2$	10	62	88
PL-1244	ReO[V]	D-Phe-Gly-L-Arg-L-Cys-L-Trp-NH$_2$	10	46	69
PL-1245	ReO[V]	D-Phe-Gly-D-Arg-L-Cys-L-Trp-NH$_2$	10	50	74
PL-1248	ReO[V]	Ac-L-Nle-D-Arg-L-Ala-D-Nal-2-L-Cys-L-Trp-NH$_2$	10	69	63
PL-1249	ReO[V]	BAla-L-Nle-L-Ala-D-Nal 2-L-Cys-L-Trp-NH$_2$	10	64	40
PL-1250	ReO[V]	BAla-D-Nle-L-Ala-D-Nal 2-L-Cys-L-Trp- NH$_2$	1	79	10
PL-1251	ReO[V]	Ac-L-Nle-L-Arg-L-Phe-D-Nal 2-L-Cys-L-Trp-NH$_2$	1	82	77
PL-1252	ReO[V]	D-Lys(Z)-L-Ala-D-Nal 2-L-Cys-L-Trp- NH$_2$	10	44	34
PL-1253	ReO[V]	L-Ala-L-Ala-D-Nal 2-L-Cys-L-Trp- NH$_2$	10	54	27
PL-1254	ReO[V]	L-Ala-D-Nal 2-L-Cys-L-Trp- NH$_2$	10	40	43
PL-1255	ReO[V]	Bz-L-Arg-L-Ala-D-Nal-2-L-Cys-L-Trp-NH$_2$	1	85	14
PL-1256	ReO[V]	L-Arg-D-Nal 2-L-Cys-L-Trp- NH$_2$	10	60	57
PL-1257	ReO[V]	HOOC(CH$_2$)$_2$-CO-L-Arg-D-Nal 2-L-Cys-L-Trp-NH$_2$	10	51	43
PL-1258	ReO[V]	BAla-L-Nle-D-Nal-2-L-Cys-L-Trp-NH$_2$	10	66	57
PL-1259	ReO[V]	Ac-L-Nle-L-Ala-L-His-D-Phe-L-Arg-L-Cys- NH$_2$	1	31	82
PL-1260	ReO[V]	Ac-L-Nle-L-Arg-L-Ala-D-Nal 2-L-Cys- NH$_2$	1	74	49
PL-1261	ReO[V]	Heptanoyl-L-His-D-Phe-L-Arg-L-Cys-L-Trp-NH$_2$	1	30	101
PL-1262	ReO[V]	Heptanoyl-D-Phe-L-Arg-L-Cys-L-Trp- NH$_2$	1	20	44
PL-1263	ReO[V]	Ac-L-Arg(Tos)-D-Phe-L-Arg-L-Cys-L-Trp- NH$_2$	1	26	33
PL-1264	ReO[V]	Ac-L-Nle-L-Glu-L-His-D-Nal 2-L-Arg-L-Trp-L-Cys- NH$_2$	0.1	54	45
PL-1265	ReO[V]	Ac-L-Glu-L-His-Gly-L-Arg-L-Trp-L-Cys- NH$_2$	1	67	34
PL-1266	ReO[V]	Ac-L-Nle-L-Ala-D-Trp-D-Nal 2-L-Arg-L-Trp-L-Cys- NH$_2$	1	87	30
PL-1267	ReO[V]	Admentoyl-Gly-D-Nal 2'-L-Arg-L-Trp-L-Cys-NH$_2$	1	55	36
PL-1268	ReO[V]	Bz-L-His-Gly-D-Nal 2'-L-Arg-L-Trp-L-Cys- NH$_2$	1	97	57
PL-1269	ReO[V]	Bz-L-His-L-Cys-D-Nal 2-L-Arg-L-Trp- NH$_2$	1	99	25
PL-1270	Linear	Ac-L-Nle-L-Ala-L-His-D-Phe-L-Arg-L-Cys-L-Trp- NH$_2$	1	57	101
PL-1271	ReO[V]	Ac-D-Trp-D-Arg-L-Nal 2'-L-Cys-D-His-L-Nal 2'-NH$_2$	1	32	35
PL-1272	Linear	Heptanoyl-L-His-D-Phe-L-Arg-L-Cys-L-Trp-NH$_2$	1	36	100
PL-1273	ReO[V]	Ac-L-Nle-L-Arg-D-Ala-D-Nal 2-L-Cys-L-Trp-NH$_2$	10	53	78
PL-1274	R O[V]	Ac-L-Nle-L-Arg-D-Phe-D-Nal 2-L-Cys-L-Trp-NH$_2$	10	45	75
PL-1275	ReO[V]	Des-aminoTyr-L-Lys-L-Ala-D-Nal 2-L-Cys-L-Trp- NH$_2$	1	17.	-4

TABLE 1					
Melanocortin Receptor Screening Results: Receptor Binding Assay				% Inhibition	
Com-pound ID	Metal ion / Linear peptide	Sequence Structure	Conc. Cut off (µM)	MC4-R	MC1-R (B-16)
PL-1276	ReO[V]	Heptanoyl-L-Lys-L-Ala-D-Nal 2-L-Cys-L-Trp-NH$_2$	1	64	4
PL-1277	ReO[V]	3-Pyridine propionyl--L-Lys-L-Ala-D-Nal 2-L-Cys-L-Trp- NH$_2$	1	38	-17
PL-1278	ReO[V]	EtOOC-(CH$_2$)$_4$-CO-L-Lys-L-Ala-D-Nal 2-L-Cys-L-Trp-NH$_2$	1	32	-13
PL-1279	ReO[V]	(s)-2-OH-isocaproyl--L-Lys-L-Phe-D-Nal 2-L-Cys-L-Trp- NH$_2$	1	55	-10
PL-1280	ReO[V]	4-MePhenoxyacetyl--L-Lys-L-Ala-D-Nal 2-L-Cys-L-Trp- NH$_2$	1	49	-11
PL-1281	ReO[V]	Heptanoyl-L-Lys-L-Ala-D-Phe-L-Cys-L-Trp-NH$_2$	1	29	60
PL-1282	ReO[V]	Heptanoyl-L-Lys-L-Ala-D-Phe-D-Cys-L-Trp-NH$_2$	1	14	-30
PL-1283	ReO[V]	Des-aminoPhe-L-Lys-L-Ala-D-Nal 2-L-Cys-L-Trp- NH$_2$	1	45	-20
PL-1284	ReO[V]	BAla-L-Lys(Ac)-L-Ala-D-Nal 2-L-Cys-L-Trp-NH$_2$	1	27	-26
PL-1285	ReO[V]	Ac-L-Nle-L-Ala-D-Trp-D-Phe-L-Arg-L-Trp-L-Cys- NH$_2$	1	32	54
PL-1286	ReO[V]	Ac-L-Nle-L-Ala-D-Nal 2-D-Phe-L-Arg-L-Trp-L-Cys- NH$_2$	1	34	7
PL-1287	ReO[V]	Ac-L-Nle-L-Ala-D-His-D-Phe-L-Arg-L-Trp-L-Cys- NH$_2$	1	40	62
PL-1288	ReO[V]	EtOOC-(CH$_2$)$_4$-CO-D-Phe-L-Arg-L-Cys-L-Trp-NH$_2$	1	16	-3
PL-1289	ReO[V]	4-n-heptanoyl-D-Phe-L-Arg-L-Cys-L-Trp- NH$_2$	1	31	-7
PL-1290	ReO[V]	Des-aminoTyr-D-Phe-L-Arg-L-Cys-L-Trp- NH$_2$	1	24	-22
PL-1291	ReO[V]	Me2-CH-CH(L-OH)-CO-D-Phe-L-Arg-L-Cys-L-Trp- NH$_2$	1	29	1
PL-1292	ReO[V]	Ac-D-Ala-L-HisL-Cys-D-Nal2-L-Arg-Tryptamide	1	87	25
PL-1293	ReO[V]	Ac-D-Ala-L-HisL-Cys-D-Nal2-L-Arg-Tryptamide	1	96	21
PL-1294	ReO[V]	Bz-L-His-L-Cys-D-Phe-L-Arg-L-Trp- NH$_2$	1	65	62
PL-1295	ReO[V]	Bz-L-Ala-L-Cys-D-Phe-L-Arg-L-Trp- NH$_2$	1	72	62
PL-1296	ReO[V]	Bz-L-Nal 2-L-Cys-D-Phe-L-Arg-L-Trp- NH$_2$	1	51	51
PL-1297	ReO[V]	Des-aminoPhe-L-Cys-D-Nal 2-L-Arg-L-Trp-NH$_2$	1	91	31
PL-1298	ReO[V]	Heptanoyl-L-Cys-D-Nal 2-L-Arg-L-Trp- NH$_2$	1	95	43
PL-1299	ReO[V]	Heptanoyl-BAla-L-Arg-L-Cys-L-Trp- NH$_2$	1	19	1
PL-1300	ReO[V]	Heptanoyl-L-Ala-L-Arg-L-Cys-L-Trp- NH$_2$	1	22	62
PL-1301	ReO[V]	L-Z-Lys-L-Ala-D-Nal 2-L-CysL-Trp- NH$_2$	1	72	49
PL-1302	ReO[V]	Heptanoyl-L-His-D-Phe-L-Arg-D-CysL-Trp-NH$_2$	1	24	77
PL-1303	ReO[V]	Heptanoyl-L-His-D-Nal 2-L-Arg-D-CysL-Trp-NH$_2$	1	60	39
PL-1304	ReO[V]	D-Z-Lys-L-Ala-D-Nal 2-L-Cys-L-Trp- NH$_2$	1	42	-10
PL-1305	ReO[V]	Ac-L-Nle-L-Arg-L-Ala-D-Nal 2-L-Cys-L-Trp-NH$_2$	1	91	14
PL-1306	ReO[V]	Ac-L-Nle-L-Arg-L-Ala-D-Phe-L-Cys-L-Trp- NH$_2$	1	36	56
PL-1309	ReO[V]	Heptanoyl-L-Cys-L-Arg-L-Cys-L-Trp- NH$_2$	1	10	9
PL-1310	ReO[V]	Des-aminoTyr-L-Lys-L-Ala-D-Nal 2-L-Cys-L-Trp- NH$_2$	10	78	100

TABLE 1 Melanocortin Receptor Screening Results: Receptor Binding Assay				% Inhibition	
Compound ID	Metal ion / Linear peptide	Sequence Structure	Conc. Cut off (µM)	MC4-R	MC1-R (B-16)
PL-1311	ReO[V]	Heptanoyl-L-Lys-L-Ala-D-Nal 2-L-Cys-L-Trp-NH$_2$	10	61	40
PL-1312	ReO[V]	3-Pyridine propionyl-L-Lys-L-Ala-D-Nal 2-L-Cys-L-Trp- NH$_2$	10	71	43
PL-1313	ReO[V]	Heptanoyl-L-Ala-L-Trp-D-Arg-L-Cys-L-Phe-NH$_2$	1	6	16
PL-1314	ReO[V]	C$_{11}$H$_{23}$-CO-L-Trp-D-Arg-L-Cys-L-Phe-NH$_2$	10	85	53
PL-1315	ReO[V]	Ac-L-Arg-L-Trp-L-Nle-L-Cys-L-Phe- NH$_2$	10	70	69
PL-1316	ReO[V]	Ac-L-Arg-D-Trp-L-Nle-L-Cys-L-Phe- NH$_2$	10	82	91
PL-1317	ReO[V]	Ac-L-Trp-L-Nle-D-Phe-L-Cys-L-Arg- NH$_2$	10	79	92
PL-1318	ReO[V]	C$_6$H$_5$-(CH$_2$)$_2$-CO-L-Nle-D-Trp-L-Cys-L-Arg-NH$_2$	10	82	82
PL-1319	ReO[V]	Ac-L-Trp-D-Arg-L-Phe-L-Cys-L-Nle-NH$_2$	10	61	89
PL-1320	ReO[V]	C$_6$H$_5$-(CH$_2$)$_2$-CO-L-Arg-L-Trp-L-Cys-L-Nle-\NH$_2$	10	94	96
PL-1321	ReO[V]	Bz-L-Arg-L-Ala-D-Phe-L-Cys-L-Phe-NH$_2$	10	79	93
PL-1322	ReO[V]	Bz-L-Arg-L-Ala-D-Trp-L-Cys-L-Phe-NH$_2$	10	66	81
PL-1323	ReO[V]	Bz-L-Arg-L-Ala-D-Phe-L-Cys-L-Nle-NH$_2$	10	77	99
PL-1324	ReO[V]	Des-aminoPhe-L-Lys-L-Arg-D-Phe-L-Cys-L-Nle- NH$_2$	10	75	93
PL-1325	ReO[V]	Des-aminoPhe-L-Cys-D-Phe-L-Arg-L-Phe-NH$_2$	10	78	94
PL-1326	ReO[V]	Heptanoyl-D-Phe-L-Arg-D-Trp-L-Cys-NH$_2$	10	62	68
PL-1327	ReO[V]	Heptanoyl-L-Phe-L-Arg-D-Trp-L-Cys-NH$_2$	10	87	93
PL-1328	ReO[V]	Ac-D-Nal 2-L-Ala-L-Arg-L-Cys-L-Trp-NH$_2$	10	78	83
PL-1329	ReO[V]	Ac-L-Nle-L-Ala-D-Trp-D-Nal 2-L-Arg-D-Trp-L-Cys- NH$_2$	1	88	30
PL-1330	ReO[V]	Ac-L-Nle-L-Arg-L-Ala-D-Nal 2-L-Cys-D-Trp-NH$_2$	1	94	53
PL-1331	ReO[V]	Des-aminoPhe-L-Cys-D-Phe-L-Arg-L-Nle-NH$_2$	10	74	82
PL-1332	ReO[V]	Des-aminoPhe-L-Cys-D-Nal 2-L-Arg-Gly-L-Phe- NH$_2$	10	98	80
PL-1333	ReO[V]	Des-aminoPhe-L-Cys-D-Nal 2-L-Arg-Gly-L-Trp- NH$_2$	10	95	77
PL-1334	ReO[V]	C$_6$H$_5$-(CH$_2$)$_2$-CO-L-Nle-L-Trp-L-Cys-L-Arg- NH$_2$	1	12	26
PL-1335	ReO[V]	Heptanoyl-L-HPhe-D-Nal 2-L-Arg-L-Trp-L-Cys-NH$_2$	1	98	36
PL-1340	ReO[V]	D-(N-Bzl)Nal 2-L-Arg-L-Trp-L-Cys- NH$_2$	1	99	34
PL-1341	ReO[V]	D-(N-PhEt)Nal 2-L-Arg-L-Trp-L-Cys- NH$_2$	1	100	46
PL-1342	ReO[V]	Ac-L-Nle-L-Arg-D-His-D-Phe-L-Cys-L-Trp- NH$_2$	1	37	89
PL-1343	ReO[V]	Ac-L-Nle-L-Arg-L-His-D-Phe-L-Cys-L-Trp- NH$_2$	10	50	86
PL-1344	ReO[V]	Heptanoyl-L-Arg-L-Phe-L-His-L-Cys-L-Trp-NH$_2$	10	81	89
PL-1345	ReO[V]	Heptanoyl-L-Arg-L-Phe-D-His-L-Cys-L-Trp-NH$_2$	10	80	95
PL-1345	ReO[V]	Heptanoyl-L-Arg-L-Phe-D-His-L-Cys-L-Trp-NH$_2$	1	52	38
PL-1346	ReO[V]	Ph(CH$_2$)$_2$-CO-L-His-L-Arg-L-Cys-L-Trp- NH$_2$	10	77	86
PL-1347	ReO[V]	Ph(CH$_2$)$_2$-CO-D-His-L-Arg-L-Cys-L-Trp- NH$_2$	10	70	64
PL-1348	ReO[V]	Ac-L-Arg-L-His-L-Phe-L-Cys-L-Trp- NH$_2$	10	51	57
PL-1349	ReO[V]	Ac-L-Arg-D-His-L-Phe-L-Cys-L-Trp- NH$_2$	10	46	60
PL-1350	ReO[V]	Ac-L-Arg-D-His-D-Phe-L-Cys-L-Trp- NH$_2$	10	42	70
PL-1351	ReO[V]	Ac-L-Arg-L-His-D-Phe-L-Cys-L-Trp- NH$_2$	1	6	68

TABLE 1 Melanocortin Receptor Screening Results: Receptor Binding Assay				% Inhibition	
Com-pound ID	Metal ion / Linear peptide	Sequence Structure	Conc. Cut off (µM)	MC4-R	MC1-R (B-16)
PL-1358	ReO[V]	Ac-L-Nle-L-Arg-L-Trp-D-Phe-L-Cys-L-His- NH_2	10	66	98
PL-1359	ReO[V]	Ac-L-Nle-L-Arg-D-Trp-D-Phe-L-Cys-L-His- NH_2	10	62	90
PL-1360	ReO[V]	Ac-L-Arg-L-Trp-L-Phe-L-Cys-L-His- NH_2	10	62	57
PL-1362	ReO[V]	Ac-L-Arg-L-Trp-D-Phe-L-Cys-L-His- NH_2	10	59	74
PL-1363	ReO[V]	Ac-L-Arg-D-Trp-D-Phe-L-Cys-L-His- NH_2	10	74	92
PL-1364	ReO[V]	Ac-L-Trp-L-Phe-L-His-L-Cys-L-Arg- NH_2	10	72	74
PL-1365	ReO[V]	Ac-L-Trp-L-Phe-D-His-L-Cys-L-Arg- NH_2	10	64	71
PL-1366	ReO[V]	Ac-L-His-L-Phe-L-Trp-L-Cys-L-Arg- NH_2	10	64	78
PL-1367	ReO[V]	Ac-L-His-L-Phe-D-Trp-L-Cys-L-Arg- NH_2	10	73	95
PL-1370	ReO[V]	Ac-L-Nle-L-Arg-L-Ala-D-Phe(4-CF3)-L-Cys-L-Trp- NH_2	1	87	51
PL-1371	ReO[V]	Ac-L-Nle-L-Arg-L-Ala-D-Phe(3,4 di-OMe)-L-Cys-L-Trp- NH_2	1	44	35
PL-1372	ReO[V]	Ac-L-Nle-L-Arg-L-Ala-D-Phe(4-Me)-L-Cys-L-Trp- NH_2	1	87	82
PL-1373	ReO[V]	Ac-L-Nle-L-Arg-L-Ala-D-Phe(3,4 di-Cl)-L-Cys-L-Trp-NH_2	1	92	54
PL-1374	ReO[V]	Ac-L-Nle-L-Arg-L-Ala-D-Phe(4-Cl)-L-Cys-L-Trp- NH_2	1	89	83
PL-1375	ReO[V]	Ac-L-Nle-L-Arg-L-Ala-D-Phe(3,4 di-F)-L-Cys-L-Trp- NH_2	1	54	78
PL-1376	ReO[V]	Ac-L-Nle-L-Arg-L-Ala-D-Val-L-Cys-L-Trp- NH_2	1	31	33
PL-1385	ReO[V]	Ac-L-Nle-L-Arg-L-Ala-D-Leu-L-Cys-L-Trp- NH_2	1	45	34
PL-1386	ReO[V]	HOOC-$(CH_2)_5$-CO-L-His-D-Phe-L-Arg-L-Cys-L-Trp- NH_2	0.1	1	71
PL-1387	Linear	HOOC-$(CH_2)_5$-CO-L-His-D-Phe-L-Arg-L-Cys-L-Trp- NH_2	0.1	1	76
PL-1388	ReO[V]	NH_2-$(CH_2)_5$-CO-L-His-D-Phe-L-Arg-L-Cys-L-Trp- NH_2	0.1	-10	80
PL-1389	Linear	NH_2-$(CH_2)_5$-CO-L-His-D-Phe-L-Arg-L-Cys-L-Trp- NH_2	0.1	7	87
PL-1390	ReO[V]	Z-L-Lys-D-His-D-Nal 2-L-Cys-L-Trp- NH_2	10	63	88
PL-1391	ReO[V]	Ac-L-Nle-L-Arg-D-His-D-Nal 2-L-Cys-L-Trp-NH_2	10	72	103
PL-1391	ReO[V]	Ac-L-Nle-L-Arg-D-His-D-Nal 2-L-Cys-L-Trp-NH_2	1	25	58
PL-1392	ReO[V]	B Ala-D-Nle-D-His-D-Nal 2-L-Cys-L-Trp- NH_2	10	80	84
PL-1393	ReO[V]	Bz-L-Arg-D-His-D-Nal 2-L-Cys-L-Trp- NH_2	10	65	86
PL-1394	ReO[V]	Ac-L-His-L-Arg-L-Trp-L-Cys-L-Phe- NH_2	10	59	84
PL-1394	ReO[V]	Ac-L-His-L-Arg-L-Trp-L-Cys-L-Phe- NH_2	1	0	27
PL-1395	ReO[V]	Ac-L-His-D-Arg-L-Trp-L-Cys-L-Phe- NH_2	1	5	10
PL-1395	ReO[V]	Ac-L-His-D-Arg-L-Trp-L-Cys-L-Phe- NH_2	10	78	74
PL-1396	ReO[V]	Ac-L-Nle-L-Ala-L-His-D-Nal 2-L-Arg-L-Cys-L-Trp- NH_2	1	70	96
PL-1405	ReO[V]	Heptanoyl-L-Phe-L-His-L-Cys-L-Trp- NH_2	1	27	20
PL-1406	ReO[V]	Heptanoyl-L-Arg-L-Phe-L-His-L-Cys-L-Trp-NH_2	1	42	32
PL-1412	ReO[V]	L-Phe-L-His-L-Cys-L-Trp- NH_2	1	54	43
PL-1413	ReO[V]	Heptanoyl-D-Nal 2'-L-Arg-L-Trp-L-Cys- NH_2	1	86	51
PL-1414	ReO[V]	Heptanoyl-Psi D-Nal 2'-L-Arg-L-Trp-L-Cys-NH_2	1	53	26

TABLE 1 Melanocortin Receptor Screening Results: Receptor Binding Assay				% Inhibition	
Com-pound ID	Metal ion / Linear peptide	Sequence Structure	Conc. Cut off (µM)	MC4-R	MC1-R (B-16)
PL-1415	ReO[V]	Heptanoyl-D-Nal 2'-Psi L-Arg-L-Trp-L-Cys-NH₂	1	0	25
PL-1416	ReO[V]	Ac-L-Nle-L-Ala-L-His-L-Phe-L-Arg-L-Cys-L-Trp- NH₂	1	35	85
PL-1417	ReO[V]	Ac-L-Nle-L-Ala-L-His-L-Phe-D-Arg-L-Cys-L-Trp- NH₂	1	11	20
PL-1418	ReO[V]	Ac-L-Nle-L-Ala-L-His-D-Phe-D-Arg-L-Cys-L-Trp- NH₂	1	24	80
PL-1419	ReO[V]	Ac-L-Nle-L-Ala-L-His-D-Nal 2'-L-Arg-L-Cys-L-Trp- NH₂	1	51	70
PL-1420	ReO[V]	Ac-L-Nle-L-Ala-L-His-L-Nal 2'-D-Arg-L-Cys-L-Trp- NH₂	1	52	43
PL-1421	ReO[V]	Ac-L-Nle-L-Ala-L-His-D-Nal 2'-D-Arg-L-Cys-L-Trp- NH₂	1	59	81
PL-1422	ReO[V]	Ac-L-Nle-L-Arg-L-Ala-D-Phe(4-Cl)-L-Cys-L-His- NH₂	1	63	69
PL-1423	ReO[V]	Ac-L-Nle-L-Arg-L-Ala-D-Phe(4-Cl)-L-Cys L-Phe(4-NO₂)- NH₂	1	71	78
PL-1424	ReO[V]	Ac-L-Nle-L-Arg-L-Ala-D-Phe(4-Cl)-L-Cys-L-Phe- NH₂	1	66	85
PL-1425	ReO[V]	Ac-L-Nle-L-Arg-L-Ala-D-Phe(4-Cl)-L-Cys-L-Glu- NH₂	1	6	39
PL-1426	ReO[V]	Ac-L-Nle-L-Arg-L-Ala-D-Phe(4-Cl)-L-Cys-L-Gln- NH₂	1	16	71
PL-1427	ReO[V]	Ac-L-Nle-L-Arg-L-Ala-D-Phe(4-Cl)-L-Cys-L-Trp- NH₂	1	82	90
PL-1428	ReO[V]	Ac-L-Nle-L-Arg-L-Ala-D-His-L-Cys-L-Trp- NH₂	1	45	56
PL-1429	ReO[V]	Ac-L-Nle-L-Arg-L-Ala-D-Phe(4-Me)-L-Cys-L-Trp- NH₂	1	84	80
PL-1430	ReO[V]	Ac-L-Nle-L-Arg-L-Ala-D-HomoPhe-L-Cys-L-Trp- NH₂	1	40	26
PL-1431	ReO[V]	Ac-L-Nle-L-Arg-L-Ala-D-Phg-L-Cys-L-Trp- NH₂	1	41	20
PL-1432	ReO[V]	Ac-L-Nle-L-Arg-L-Ala-D-Trp-L-Cys-L-Trp- NH₂	1	47	38
PL-1433	ReO[V]	Ac-L-Nle-L-Arg-L-Ala-D-Cha-L-Cys-L-Trp- NH₂	1	38	14
PL-1434	ReO[V]	Ac-L-Nle-L-Arg-L-Ala-D-Chg-L-Cys-L-Trp- NH₂	1	53	33
PL-1435	ReO[V]	Ac-L-Nle-L-Arg-L-His-L-Ala-D-Phe(4-Cl)-L-Cys-L-Trp- NH₂	1	59	44
PL-1436	ReO[V]	Ac-L-Nle-L-His-L-Arg-L-Ala-D-Phe(4-Cl)-L-Cys-L-Trp- NH₂	1	58	51
PL-1438	ReO[V]	Ac-L-Nle-L-Arg-L-His-D-Phe(4-Cl)-L-Cys-L-Trp- NH₂	1	91	89
PL-1439	ReO[V]	Ac-L-Nle-L-Arg-D-His-D-Phe(4-Cl)-L-Cys-L-Trp- NH₂	1	43	92
PL-1440	ReO[V]	Ac-L-Nle-L-Arg-L-Lys-D-Phe(4-Cl)-L-Cys-L-Trp- NH₂	1	94	79
PL-1441	ReO[V]	Ac-L-Nle-L-Arg-D-Lys-D-Phe(4-Cl)-L-Cys-L-Trp- NH₂	1	51	71
PL-1442	ReO[V]	Ac-L-Nle-L-Arg-L-Ala-D-Phe(4-F)-L-Cys-L-Trp-NH₂	1	85	93
PL-1443	ReO[V]	Ac-L-Nle-L-Arg-L-Ala-D-Phe(4-I)-L-Cys-L-Trp-NH₂	1	99	72
PL-1444	ReO[V]	Ac-L-Nle-L-Arg-L-Ala-D-Tyr-L-Cys-L-Trp- NH₂	1	52	68

		TABLE 1 Melanocortin Receptor Screening Results: Recept r Binding Assay		% Inhibition	
Com-pound ID	Metal ion / Linear peptide	Sequence Structure	Conc. Cut off (µM)	MC4-R	MC1-R (B-16)
PL-1445	ReO[V]	Ac-L-Nle-L-Arg-L-Ala-D-Phe(4-NO₂)-L-Cys-L-Trp- NH₂	1	83	63
PL-1446	ReO[V]	L-Ala-D-Nal 2'-L-Cys-L-Trp- NH₂	1	50	14
PL-1447	ReO[V]	Heptanoyl-L-Ala-D-Nal 2'-L-Cys-L-Trp- NH₂	1	44	12
PL-1448	ReO[V]	L-Arg-D-Nal 2'-L-Cys-L-Trp- NH₂	1	62	26
PL-1449	ReO[V]	Heptanoyl-L-Arg-D-Nal 2'-L-Cys-L-Trp- NH₂	1	80	45
PL-1450	ReO[V]	Ac-L-Nle-L-Ala-L-His-L-Phe-L-Arg-L-Cys-L-Trp- NH₂	1	66	73
PL-1451	ReO[V]	Ac-L-Nle-L-Arg-L-Ala-D-Phe(4-Br)-L-Cys-L-Trp- NH₂	1	92	77
PL-1452	ReO[V]	Ac-L-Nle-L-Arg-L-Ala-D-Phe(4-Br)-L-Cys-L-Trp- NH₂	1	97	86
PL-1457	ReO[V]	Ac-L-Ala-L-His-L-Cys-D-Bip-L-Arg-L-Trp- NH₂	1	79	26
PL-1458	ReO[V]	Ac-L-Ala-L-His-L-Cys-D-Phe-L-Arg-L-Trp- NH₂	1	61	48
PL-1459	ReO[V]	Ac-L-Ala-L-His-L-Cys-D-Asp(3Cl-anilino)-L-Arg-L-Trp- NH₂	1	31	68
PL-1460	ReO[V]	Ac-L-Ala-L-His-L-Cys-D-Asp(3,5 diCl-anilino)-L-Arg-L-Trp-NH₂	1	40	47
PL-1461	ReO[V]	Ac-L-Ala-L-His-L-Cys-D-Asp(anilino)-L-Arg-L-Trp- NH₂	1	60	43
PL-1462	ReO[V]	Heptanoyl-L-His-D-Phe-L-Arg-L-Cys-L-Nal 2'-NH₂	1	39	99
PL-1463	ReO[V]	Heptanoyl-L-His-D-Phe-L-Arg-D-Cys-L-Nal 2'-NH₂	1	57	93
PL-1464	ReO[V]	Heptanoyl-L-His-D-Phe-L-Arg-L-Cys-D-Nal 2'-NH₂	1	80	94
PL-1465	ReO[V]	Heptanoyl-L-His-D-Phe-L-Arg-D-Cys-D-Nal 2'-NH₂	1	48	84
PL-1466	ReO[V]	Heptanoyl-L-His-D-Phe-D-Arg-L-Cys-L-Trp-NH₂	1	37	67
PL-1467	ReO[V]	Heptanoyl-L-His-D-Phe-D-Arg-D-Cys-L-Trp-NH₂	1	47	69
PL-1478	ReO[V]	Ac-L-Arg-L-Phe-L-Phe-L-Cys-L-Ser- NH₂	1	21	28
PL-1480	ReO[V]	Ac-L-Arg-D-Phe-L-Phe-L-Cys-L-Ser- NH₂	1	9	21
PL-1481	ReO[V]	Ac-L-Arg-D-Phg-L-Phe-L-Cys-L-Ser- NH₂	1	15	19
PL-1483	ReO[V]	Ac-L-Arg-L-Phe-L-Nal 2'-L-Cys-L-Ser- NH₂	1	31	56
PL-1484	ReO[V]	Ac-L-Arg-D-Phe-L-Nal 2'-L-Cys-L-Ser- NH₂	1	37	72
PL-1485	ReO[V]	Ac-L-Arg-D-Phg-L-Nal 2'-L-Cys-L-Ser- NH₂	1	41	60
PL-1486	ReO[V]	Ac-D-Ala-L-His-L-Cys-D-Nal 2-L-Arg-L-Trp-NH₂	1	90	26
PL-1488	Linear	Ac-D-Ala-L-His-L-Cys-D-Nal 2-L-Arg-L-Trp-NH₂	1	85	35
PL-1489	ReO[V]	Heptanoyl-L-Ser(Bzl)-D-Nal 2'-L-Arg-L-Trp-L-Cys- NH₂	1	99	30
PL-1490	Linear	Heptanoyl-L-Ser(Bzl)-D-Nal 2'-L-Arg-L-Trp-L-Cys- NH₂	1	86	48
PL-1491	ReO[V]	Heptanoyl-L-Ser(Bzl)-D-Nal 2'-L-Arg-L-Phe-L-Cys- NH₂	1	76	66
PL-1492	ReO[V]	Heptanoyl-L-Ser(Bzl)-D-Nal 2'-L-Arg-D-Phe-L-Cys- NH₂	1	90	63
PL-1493	ReO[V]	Heptanoyl-L-Ser(Bzl)-D-Nal 2'-L-Arg-D-Trp-L-Cys- NH₂	1	102	26

TABLE 1 Melanocortin R eptor Screening R sults: R c ptor Binding Assay				% Inhibition	
Com-pound ID	M tal ion / Linear peptide	Sequence Structure	Conc. Cut off (μM)	MC4-R	MC1-R (B-16)
PL-1494	ReO[V]	Ac-L-Lys-L-Cys-D-Phe-L-Trp-L-Nle- NH$_2$	1	30	27
PL-1496	ReO[V]	Ac-L-Asp-L-Lys-L-Pro-L-Pro-L-Arg-L-Ala-D-Nal 2'-L-Cys-L-Trp- NH$_2$	1	59	43
PL-1497	ReO[V]	L-Asp-L-Lys-L-Pro-L-Pro-L-Arg-L-Ala-D-Nal 2'-L-Cys-L-Trp- NH$_2$	1	71	47
PL-1498	ReO[V]	Ac-L-Nle-L-Arg-L-Ala-D-Nal 2'-L-Cys-L-Trp-Ahx-L-Lys-L-Asp- NH$_2$	1	86	59
PL-1499	ReO[V]	Heptanoyl-L-Lys-D-Phe-L-Trp-L-Cys- NH$_2$	1	20	23
PL-1500	ReO[V]	Heptanoyl-D-Phe-L-Trp-L-Cys-L-Lys- NH$_2$	1	33	25
PL-1501	ReO[V]	Heptanoyl-L-His-D-Trp-L-Cys-L-Trp- NH$_2$	1	44	78
PL-1502	ReO[V]	Heptanoyl-L-His-D-Trp-Gly-L-Cys-L-Lys- NH$_2$	1	41	29
PL-1503	ReO[V]	Heptanoyl-L-Phe-D-Phe-L-Arg-L-Cys-L-Trp-NH$_2$	1	41	52
PL-1504	ReO[V]	Ac-L-Arg-L-Phe-L-Nal 2'-L-Asn-L-Cys-L-Phe-NH$_2$	1	21	36
PL-1505	ReO[V]	Ac-L-Arg-L-Phe-L-Nal 2'-L-Asn-L-Cys-L-Phe-NH$_2$	1	29	45
PL-1506	ReO[V]	Ac-L-Arg-D-Phe-L-Nal 2'-L-Asn-L-Cys-L-Phe-NH$_2$	1	22	30
PL-1507	ReO[V]	Ac-L-Arg-L-Phe-L-Phe-L-Asn-L-Cys-L-Phe-NH$_2$	1	22	20
PL-1508	ReO[V]	Ac-L-Arg-D-Phe-L-Phe-L-Asn-L-Cys-L-Phe-NH$_2$	1	31	20
PL-1509	ReO[V]	Ac-L-Arg-L-Phe-L-Cys-L-Phe-L-Asn-L-Ala-L-Phe- NH$_2$	1	30	19
PL-1510	ReO[V]	Ac-L-Arg-L-Phe-L-Phe-L-Cys-L-Asn-L-Ala-L-Phe- NH$_2$	1	31	21
PL-1511	ReO[V]	Ac-L-Arg-L-Phe-L-Phe-L-Asn-L-Cys-L-Ala-L-Phe- NH$_2$	1	26	21
PL-1512	ReO[V]	Ac-L-Arg-L-Phe-L-Phe-L-Asn-L-Ala-L-Cys-L-Phe- NH$_2$	1	24	20
PL-1513	ReO[V]	Ac-L-Arg-L-Phe-L-Phe-L-Asn-L-Phe-L-Cys-NH$_2$	1	18	23
PL-1514	ReO[V]	Ac-L-Arg-L-Phe-L-Phe-L-Asn-L-Phe-L-Cys-NH$_2$	1	30	28
PL-1515	ReO[V]	Heptanoyl-L-His-D-Phe-L-Lys-L-Cys-L-Glu	1	34	21
PL-1518	ReO[V]	Heptanoyl-L-Cys-D-Phe-L-Trp-L-Lys- NH$_2$	1	25	8
PL-1519	ReO[V]	Ac-L-Nle-L-Arg-L-Ala-D-Nal 2'-L-Cys-L-Trp-L-Pro-L-Pro-L-Lys-L-Asp-NH$_2$	1	60	22
PL-1522	ReO[V]	Ac-L-Nle-L-Arg-L-His-D-Phe(Br-4')-L-Cys-L-Trp-NH$_2$	1	91	87
PL-1523	ReO[V]	Ac-L-Nle-L-Arg-L-Ala-L-His-D-Phe(Br-4')-L-Cys-L-Trp-NH$_2$	1	59	69
PL-1524	ReO[V]	Ac-L-Nle-L-Arg-L-His-L-Ala-D-Phe(Br-4')-L-Cys-L-Trp-NH$_2$	1	44	36
PL-1525	ReO[V]	Ac-L-Nle-L-Arg-L-Phe(Br-4')-L-Ala-L-His-L-Cys-L-Trp-NH$_2$	1	41	57
PL-1526	ReO[V]	Ac-L-Nle-L-Arg-L-Trp-L-Ala-D-Phe(Br-4')-L-Cys-L-His-NH$_2$	1	34	63
PL-1581	ReO[V]	Ac-L-Nle-L-Ala-L-His-D-Phe-L-Arg-L-Trp-L-Cys-NH$_2$	1	98	91

TABLE 1				% Inhibition	
Melanocortin Receptor Screening Results: Receptor Binding Assay					
Com-pound ID	Metal ion / Linear peptide	Sequence Structure	Conc. Cut off (µM)	MC4-R	MC1-R (B-16)
PL-1582	ReO[V]	Ac-L-Nle-L-Ala-L-His-D-Phe-L-Cys-L-Arg-L-Trp-NH₂	1	66	93
PL-1583	ReO[V]	Ac-L-Nle-L-Ala-L-His-L-Cys-D-Phe-L-Arg-L-Trp-NH₂	1	75	84
PL-1584	ReO[V]	Ac-L-Nle-L-His-L-Cys-L-Hid-D-Phe-L-Arg-L-Trp-NH₂	1	71	96
PL-1585	ReO[V]	Ac-L-Nle-L-Ala-L-His-L-Phe-L-Cys-L-Arg-L-Trp-NH₂	1	22	45
PL-1587	ReO[V]	Ac-L-Nle-L-Arg-L-Arg-D-Nal 2-L-Cys-L-Trp-NH₂	1	98	96
PL-1592	ReO[V]	Ac-L-Nle-L-Ala-L-Arg-L-His-D-Phe-L-Cys-L-Trp-NH₂	1	7	19
PL-1593	ReO[V]	Ac-L-Nle-L-Ala-D-Arg-L-His-D-Phe-L-Cys-L-Trp-NH₂	1	16	71
PL-1594	ReO[V]	Ac-L-Nle-L-Ala-L-His-D/L-Atc-L-Arg-L-Cys-L-Trp-NH₂	1	24	100
PL-1595	ReO[V]	Ac-L-Nle-L-Ala-L-His-Aic-L-Arg-L-Cys-L-Trp-NH₂	1	3	60
PL-1597	ReO[V]	Ac-L-Nle-L-Arg-L-Ala-D/L-Atc-L-Cys-L-Trp-NH₂	1	11	68
PL-1598	ReO[V]	Ac-L-Nle-L-Arg-L-Arg-D-Nal 2'-L-Cys-L-Trp-NH₂	1	9	22
PL-1605	ReO[V]	Ac-L-Nle-L-Ala-L-His-Achc-L-Arg-L-Cys-L-Trp-NH₂	1	100	100
PL-1606	ReO[V]	Ac-L-Nle-L-Arg-L-Ala-Aic-L-Cys-L-Trp- NH₂	1	63	44
PL-1607	ReO[V]	Ac-L-Nle-L-Ala-L-His-D-Qal 2'-L-Arg-L-Cys-L-Trp- NH₂	1	52	100
PL-1621	ReO[V]	Ac-L-Nle-L-Ala-L-His-Achc-L-Arg-L-Cys-L-Trp-NH₂	1	34	36
PL-1623	ReO[V]	Ac-L-Nle-L-Ala-L-His-D-Sal-L-Arg-L-Cys-L-Trp-NH₂	1	55	92
PL-1624	ReO[V]	Ac-L-Nle-L-Arg-L-Ala-D-Sal-L-Cys-L-Trp- NH₂	1	48	25
PL-1626	ReO[V]	Ac-L-Nle-L-Arg-L-Trp-D-Nal 2'L-Cys-L-Trp-NH₂	1	54	66
PL-1633	ReO[V]	Ac-L-Nle-D-Arg-L-Arg-D-Nal 2'L-Cys-L-Trp-NH₂	1	87	86
PL-1633	ReO[V]	Ac-L-Nle-D-Arg-L-Arg-D-Nal 2'L-Cys-L-Trp-NH₂	1	87	91
PL-1634	ReO[V]	Ac-L-Nle-L-Arg-D-Arg-D-Nal 2'L-Cys-L-Trp-NH₂	1	50	42
PL-1635	ReO[V]	Ac-L-Nle-L-Arg-L-Ala-Acpc-L-Cys-L-Trp-NH2	1	43	14
PL-1636	ReO[V]	Ac-L-Nle-L-Ala-L-His-Acpc-L-Arg-L-Cys-L-Trp-NH₂	1	38	20
PL-1638	ReO[V]	Ac-L-Nle-L-Arg-L-Arg-D-Qal 2'-L-Cys-L-Trp-NH₂	1	48	67
PL-1649	ReO[V]	Ac-L-His-Gly-Gly-L-Cys-L-Trp- NH₂	10	62	19
PL-1650	ReO[V]	Ac-L-His-D-Phe-L-Arg-L-Cys-L-Trp- NH₂	10	66	52
PL-1651	ReO[V]	Ac-L-His-D-Phe-D-Arg-L-Cys-L-Trp- NH₂	10	58	95
PL-1652	ReO[V]	Ac-L-His-L-Phe-D-Arg-L-Cys-L-Trp- NH₂	10	40	11
PL-1655	ReO[V]	Ac-L-His-L-Phe-L-Arg-L-Cys-L-Trp- NH₂	10	51	45
PL-1658	ReO[V]	Ac-L-Nle-L-Arg-L-Arg-D-Phe (3,4,di Cl)-L-Cys-L-Trp- NH₂	1	100	99

Com-pound ID	Metal ion / Linear peptide	Sequence Structure	Conc. Cut off (μM)	MC4-R	MC1-R (B-16)
TABLE 1 Melanocortin Receptor Screening Results: Receptor Binding Assay				% Inhibition	
PL-1658	ReO[V]	Ac-L-Nle-L-Arg-L-Arg-D-Phe (3,4,di Cl)-L-Cys-L-Trp- NH_2	1	97	93
PL-1659	ReO[V]	Ac-L-Nle-L-Lys-L-Lys-D-Nal 2'-L-Cys-L-Trp-NH_2	1	91	58
PL-1660	ReO[V]	Ac-L-Nle-L-Arg-L-Arg-D-Phe-L-Cys-L-Trp- NH_2	1	67	100
PL-1661	ReO[V]	Ac-L-Nle-L-Cit-L-Cit-D-Nal 2'-L-Cys-L-Trp- NH_2	1	58	27
PL-1662	ReO[V]	Ac-L-Nle-L-Ala-L-Arg-L-Arg-D-Nal 2'-L-Cys-L-Trp- NH_2	1	93	69
PL-1663	ReO[V]	Ac-L-Nle-L-Arg-L-Arg-D-Trp-L-Cys-L-Trp- NH_2	1	85	81
PL-1664	ReO[V]	Ac-L-Nle-L-Arg-L-Arg-D-Nal 2'L-Cys- NH_2	1	100	96
PL-1665	ReO[V]	Ac-L-Arg-L-Arg-D-Nal 2'-L-Cys-L-Trp- NH_2	1	93	94
PL-1666	ReO[V]	Ac-L-Nle-L-Arg-L-Arg-D-Phe(p-I)-L-Cys-L-Trp-NH_2	1	101	101
PL-1667	ReO[V]	Heptanoyl-L-Arg-L-Arg-D-Nal 2'-L-Cys-L-Trp-NH_2	1	97	92
PL-1684	ReO[V]	Ac-L-Val-L-Pro-L-Arg-L-Ala-D-Nal-2'-L-Cys-L-Trp- NH_2	1	36	26
PL-1685	ReO[V]	Ac-L-Nle-L-Arg-BAla-D-Nal-2'-L-Cys-L-Trp-NH_2	1	83	54
PL-1686	ReO[V]	Ac-L-Nle-D-Arg-D-Arg-L-Nal-2'-L-Cys-L-Trp-NH_2	1	24	23
PL-1690	ReO[V]	Ac-L-Nle-D-Arg-L-Arg-D-Nal-2'-L-Cys-L-Trp-NH_2	1	94	71
PL-1691	Linear	Heptanoyl-L-His-D-Phe-L-Arg-L-Cys-L-Trp-L-Lys- NH_2	1	90	62
PL-1692	Linear	NH_2-$(CH_2)_5$-CO-L-His-D-Phe-L-Arg-L-Cys-L-Trp-L-Lys- NH_2	1	79	60
PL-1694	ReO[V]	Heptanoyl-L-His-D-Phe-L-Arg-L-Cys-L-Trp-L-Lys- NH_2	1	43	61
PL-1695	ReO[V]	NH_2-$(CH_2)_5$-CO-L-His-D-Phe-L-Arg-L-Cys-L-Trp-L-Lys- NH_2	1	44	87
PL-1702	ReO[V]	Ac-L-His-L-Arg-L-Arg-D-Nal 2'-L-Cys- NH_2	1	83	62
PL-1703	ReO[V]	Ac-L-Trp-L-Arg-L-Arg-D-Nal 2'-L-Cys- NH_2	1	95	73
PL-1704	ReO[V]	Ac-L-Phe-L-Arg-L-Arg-D-Nal 2'-L-Cys- NH_2	1	98	75
PL-1705	ReO[V]	Ac-L-Lys-L-Arg-L-Arg-D-Nal 2'-L-Cys- NH_2	1	90	53
PL-1706	ReO[V]	Ac-L-Ser-L-Arg-L-Arg-D-Nal 2'-L-Cys- NH_2	1	89	60
PL-1707	ReO[V]	Ac-L-Glu-L-Arg-L-Arg-D-Nal 2'-L-Cys- NH_2	1	46	36
PL-1708	ReO[V]	Ac-L-Arg-L-His-L-Cys-D-Nal 2'-L-Arg-L-Trp-NH_2	1	99	34
PL-1709	ReO[V]	Ac-D-Ala-L-His-L-Cys-L-Arg-D-Nal 2'-L-Arg-L-Trp- NH_2	1	100	98
PL-1710	ReO[V]	Ac-D-Ala-L-Arg-L-Cys-D-Nal 2'-L-Arg-L-Trp-NH_2	1	99	25
PL-1718	ReO[V]	Ac-L-Nle-L-Arg-L-Ala-D-Phe(3'-Cl)-L-Cys-L-Trp- NH_2	1	59	35
PL-1722	ReO[V]	Ac-L-Trp-L-Arg-L-Arg-D-Phe-L-Cys- NH_2	1	29	38
PL-1723	ReO[V]	Ac-L-Nle-L-Arg-L-Arg-D-Nal 2'-L-Cys-OH	1	85	66
PL-1726	ReO[V]	Ac-L-Nle-L-Arg-L-Hphe-D-Phe(4'-Cl)-L-Cys-NH_2	1	70	42
PL-1727	ReO[V]	Ac-L-Nle-L-Arg-L-Pal 2'-D-Phe(4'-Cl)-L-Cys-NH_2	1	84	62
PL-1728	ReO[V]	Ac-L-Nle-L-Arg-L-Phe-D-Phe(4'-Cl)-L-Cys- NH_2	1	73	53

TABLE 1				% Inhibition	
Melanocortin Receptor Screening Results: Receptor Binding Assay					
Com-pound ID	Metal ion / Linear peptide	Sequence Structure	Conc. Cut off (µM)	MC4-R	MC1-R (B-16)
PL-1730	ReO[V]	Ac-L-Nle-L-Arg-L-Nal 1'-D-Phe(4'-Cl)-L-Cys-NH$_2$	1	35	39
PL-1731	ReO[V]	Ac-L-Nle-L-Arg-L-Nal 2'-D-Phe(4'-Cl)-L-Cys-NH$_2$	1	63	38
PL-1732	ReO[V]	Ac-L-Nle-L-Arg-L-Trp-D-Phe(4'-Cl)-L-Cys- NH$_2$	1	74	53
PL-1733	ReO[V]	L-Tic-D-Phe(4'-Cl)-L-Cys- NH$_2$	1	8	14
PL-1734	ReO[V]	L-Tic-D-Phe(4'-Cl)-L-Trp-L-Cys- NH$_2$	1	7	6
PL-1735	ReO[V]	L-Tic-D-Phe(4'-Cl)-L-Cys-L-Trp- NH$_2$	1	13	12
PL-1736	ReO[V]	Ac-D-Ala-L-His-L-Cys-D-(N-Bzl)Phe-L-Arg-L-Trp- NH$_2$	1	3	6
PL-1737	ReO[V]	Ac-D-Ala-L-His-L-Cys-L-(N-Bzl)Phe-L-Arg-L-Trp- NH$_2$	1	3	48
PL-1738	ReO[V]	Ac-D-Ala-L-His-L-Cys-D-(N-Bzl)Nal 2'-L-Arg-L-Trp- NH$_2$	1	23	13
PL-1751	ReO[V]	Ac-L-His-L-(N-2' naphalene)Phe-L-Arg-L-Trp-L-Cys-NH$_2$	1	70	78
PL-1752	ReO[V]	Ac-D-Ala-L-His-L-Cys-L-(N-2' naphalene)Phe-L-Arg-L-Trp- NH$_2$	1	5	29
PL-1753	ReO[V]	Ac-D-Ala-L-His-L-Cys-D-(N-2' naphalene)Phe-L-Arg-L-Trp- NH$_2$	1	22	50
PL-1754	ReO[V]	D-Tic-D-Phe(4'-Cl)-L-Trp-L-Cys- NH$_2$	1	7	4
PL-1755	ReO[V]	Ac-L-Arg-L-Lys-L-Phe-D-Phe(4'-Cl)-L-Cys-L-Trp- NH$_2$	1	40	48
PL-1756	ReO[V]	Ac-L-Nle-L-Lys-L-Phe-D-Phe(4'-Cl)-L-Cys-L-Trp- NH$_2$	1	63	64
PL-1757	ReO[V]	Ac-L-Arg-L-Lys-L-Leu-D-Phe(4'-Cl)-L-Cys-L-Trp- NH$_2$	1	45	38
PL-1758	ReO[V]	Ac-L-Nle-L-Lys-L-Leu-D-Phe(4'-Cl)-L-Cys-L-Trp- NH$_2$	1·	94	78
PL-1759	ReO[V]	Ac-L-Arg-L-Phe-L-Lys-D-Phe(4'-Cl)-L-Cys-L-Trp- NH$_2$	1	62	61
PL-1760	ReO[V]	Ac-L-Nle-L-Phe-L-Lys-D-Phe(4'-Cl)-L-Cys-L-Trp- NH$_2$	1	72	84
PL-1761	ReO[V]	Ac-L-Arg-L-Leu-L-Lys-D-Phe(4'-Cl)-L-Cys-L-Trp- NH$_2$	1	16	51
PL-1762	ReO[V]	Ac-L-Nle-L-Leu-L-Lys-D-Phe(4'-Cl)-L-Cys-L-Trp- NH$_2$	1	69	82
PL-1774	ReO[V]	Ac-L-Nle-L-Lys-L-Val-D-Phe(4'-Cl)-L-Cys-L-Trp- NH$_2$	1	83	79
PL-1775	ReO[V]	Ac-L-Nle-L-Lys-L-Ile-D-Phe(4'-Cl)-L-Cys-L-Trp-NH$_2$	1	78	57
PL-1776	ReO[V]	Ac-L-Nle-L-Lys-L-Nle-D-Phe(4'-Cl)-L-Cys-L-Trp- NH$_2$	1	76	33
PL-1777	ReO[V]	Ac-L-Nle-L-Lys-L-Thr-D-Phe(4'-Cl)-L-Cys-L-Trp- NH$_2$	1	79	86
PL-1778	ReO[V]	Ac-L-Nle-L-Lys-L-Tle-D-Phe(4'-Cl)-L-Cys-L-Trp- NH$_2$	1	89	60
PL-1779	ReO[V]	Ac-L-Nle-L-Lys-L-Chg-D-Phe(4'-Cl)-L-Cys-L-Trp- NH$_2$	1	85	71
PL-1780	ReO[V]	Ac-L-Nle-L-Lys-L-Cha-D-Phe(4'-Cl)-L-Cys-L-Trp- NH$_2$	1	77	34

SUBSTITUTE SHEET (RULE 26)

Com-pound ID	M tal ion / Linear peptide	Sequence Structure	Conc. Cut off (μM)	MC4-R	MC1-R (B-16)
		TABLE 1 **Melan c rtin R ceptor Scre ning Results: R c ptor Binding Assay**		**% Inhibiti n**	
PL-1781	ReO[V]	Ac-L-Nle-L-Lys-L-Trp-D-Phe(4'-Cl)-L-Cys-L-Trp- NH$_2$	1	69	66
PL-1782	ReO[V]	Ac-L-Nle-L-Lys-L-Hphe-D-Phe(4'-Cl)-L-Cys-L-Trp- NH$_2$	1	67	14
PL-1783	ReO[V]	Ac-L-Nle-L-Lys-L-Lys(Z)-D-Phe(4'-Cl)-L-Cys-L-Trp- NH$_2$	1	72	87
PL-1785	ReO[V]	Ac-D-Ala-L-His-L-Cys-D-Tic-L-Arg-L-Trp- NH$_2$	1	16	51
PL-1787	ReO[V]	Ac-D-Ala-L-His-L-Cys-D-3,3,Dip-L-Arg-L-Trp-NH$_2$	1	31	49
PL-1788	ReO[V]	Ac-L-Ala-L-His-D-Phe-L-Arg-L-Trp-L-Cys- NH$_2$	1	78	62
PL-1789	ReO[V]	Ac-L-Pro-L-Ala-L-His-D-Phe-L-Arg-L-Trp-L-Cys- NH$_2$	1	87	63
PL-1790	ReO[V]	Ac-L-Nle-L-Ala-L-His-D-Phe-L-Arg-L-Trp-L-Cys-L-Trp- NH$_2$	1	99	93
PL-1791	ReO[V]	Ac-L-Nle-L-Ala-L-His-D-Phe-L-Arg-L-Trp-L-Cys-L-Leu- NH$_2$	1	98	87
PL-1792	ReO[V]	Ac-L-Nle-L-Ala-L-His-D-Phe-L-Arg-L-Trp-L-Cys-L-Lys- NH$_2$	1	100	96
PL-1793	ReO[V]	Ac-L-Pro-L-His-D-Phe-L-Arg-D-Trp-L-Cys- NH$_2$	1	39	41
PL-1794	ReO[V]	Ac-L-Pro-L-His-D-Phe-L-Arg-D-Trp-L-Cys-D-Trp- NH$_2$	1	20	-7
PL-1795	ReO[V]	Ac-D-Tic-D-Phe-L-Arg-D-Trp-L-Cys- NH$_2$	1	85	51
PL-1796	ReO[V]	Ac-D(3,3)Bpa-D-Phe-L-Arg-D-Trp-L-Cys- NH$_2$	1	14	-7
PL-1797	ReO[V]	Ac-L-Lys-L-Leu-D-Phe(4'-Cl)-L-Cys-L-Trp- NH$_2$	1	42	49
PL-1798	ReO[V]	Heptanoyl-L-Lys-L-Leu-D-Phe(4'-Cl)-L-Cys-L-Trp- NH$_2$	1	56	68
PL-1799	ReO[V]	Ac-L-Nle-L-Lys-L-Leu-D-Phe(4'-Cl)-L-Cys- NH$_2$	1	69	70
PL-1800	ReO[V]	Ac-L-Nle-L-Lys-L-Leu-D-Phe(4'-Cl)-D-Cys-L-Trp- NH$_2$	1	35	54
PL-1801	ReO[V]	Ac-L-Nle-L-Lys-L-Leu-D-Phe(4'-Cl)-L-Cys-L-Tic- NH$_2$	1	64	89
PL-1802	ReO[V]	Ac-L-Nle-L-Lys-L-Leu-D-Phe(4'-Cl)-L-Cys-L-Tyr- NH$_2$	1	16	72
PL-1803	ReO[V]	Ac-L-Nle-L-Lys-L-Leu-D-Phe(4'-Cl)-L-Cys-L-Leu- NH$_2$	1	23	55
PL-1804	ReO[V]	Ac-L-Nle-L-Lys-L-Leu-D-Phe(4'-Cl)-L-Cys-L-Tyr(Bzl)- NH$_2$	1	47	70
PL-1805	ReO[V]	Ac-L-Nle-L-Lys-L-Leu-D-Phe(4'-Cl)-L-Cys-L-Phe(3-Cl)-NH2	1	67	74
PL-1806	ReO[V]	Ac-L-Nle-L-Ala-L-His-L-Phe-L-Arg-L-Cys-L-Trp- NH$_2$	1	13.0	66.0
PL-1807	ReO[V]	Ac-L-Nle-L-Glu-L-His-D-Phe-L-Arg-L-Cys-L-Trp- NH$_2$	1	38.0	96.0
PL-1808	ReO[V]	Ac-L-His-D-Phe-L-Arg-L-Trp-L-Cys-L-Lys-L-Pro-L-Val- NH$_2$	1	75.0	94.0
PL-1809	ReO[V]	L-Tyr-L-Val-L-Nle-Gly-L-His-L-Phe-L-Arg-L-Trp-L-Asp-L-Arg-L-Cys-L-Phe- NH$_2$	1	77.0	88.0
PL-1810	ReO[V]	L-Tyr-L-Val-L-Nl -Gly-L-His-L-Phe-L-Arg-L-Trp-L-Asp-L-Cys-L-Arg-L-Phe- NH$_2$	1	88.0	91.0
PL-1811	ReO[V]	L-Tyr-L-Val-L-Nle-Gly-L-His-L-Phe-L-Arg-L-Trp-L-Cys-L-Asp-L-Arg-L-Phe- NH$_2$	1	92.0	95.0

TABLE 1 Melancortin Receptor Screening Results: Receptor Binding Assay				% Inhibition	
Compound ID	Metal ion / Linear peptide	Sequence Structure	Conc. Cut off (µM)	MC4-R	MC1-R (B-16)
PL-1812	ReO[V]	L-Tyr-L-Val-L-Nle-Gly-L-His-L-Phe-L-Arg-L-Cys-L-Trp-L-Asp-L-Arg-L-Phe- NH$_2$	1	98.0	98.0
PL-1813	ReO[V]	L-Tyr-L-Val-L-Nle-Gly-L-His-L-Phe-L-Cys-L-Arg-L-Trp-L-Asp-L-Arg-L-Phe- NH$_2$	1	36.0	67.0
PL-1814	ReO[V]	L-Tyr-L-Val-L-Nle-Gly-L-His-L-Cys-L-Phe-L-Arg-L-Trp-L-Asp-L-Arg-L-Phe- NH$_2$	1	26.0	62.0
PL-1815	ReO[V]	L-Tyr-L-Val-L-Nle-Gly-L-Cys-L-His-Phe-L-Arg-L-Trp-L-Asp-L-Arg-L-Phe- NH$_2$	1	36.0	60.0
PL-1816	ReO[V]	Heptanoyl-L-Ser(Bzl)-D-Phe(2'-Cl)-L-Arg-D-Trp-L-Cys- NH$_2$	1	83.0	46.0
PL-1817	ReO[V]	Bz-L-His-Gly-D-Phe(2'-Cl)-L-Arg-D-Trp-L-Cys-NH$_2$	1	68.0	45.0
PL-1818	ReO[V]	Ac-L-Nle-L-Ala-D-Trp-D-Phe(2'-Cl)-L-Arg-D-Trp-L-Cys- NH$_2$	1	36.0	15.0
PL-1819	ReO[V]	Ac-L-Ala-L-His-D-Phe(2'-Cl)-L-Arg-D-Trp-L-Cys- NH$_2$	1	67.0	76.0
PL-1820	ReO[V]	Bz-D-Phe(2'-Cl)-L-Arg-D-Trp-L-Cys- NH$_2$	1	54.0	17.0
PL-1821	ReO[V]	Z-L-Lys-L-Ala-D-Phe-L-Cys-L-Trp- NH$_2$	1	18	43
PL-1822	ReO[V]	Z-L-Lys-L-Ala-D-Phe(2'-Cl)-L-Cys-L-Trp- NH$_2$	1	15	24
PL-1823	ReO[V]	Z-L-Lys-L-Ala-D-Phe(3'-Cl)-L-Cys-L-Trp- NH$_2$	1	35	11
PL-1824	ReO[V]	Z-L-Lys-L-Ala-D-Phe-(4'-Cl)-L-Cys-L-Trp- NH$_2$	1	69	34
PL-1825	ReO[V]	Z-L-Lys-L-Ala-D-Phe-(3,4,-Di-Cl)L-Cys-L-Trp-NH$_2$	1	58	15
PL-1828	ReO[V]	Heptanoyl-L-Tyr-D-Phe(2'-Cl)-L-Arg-D-Trp-L-Cys- NH$_2$	1	29	29
PL-1829	ReO[V]	Heptanoyl-L-Trp-D-Phe(2'-Cl)-L-Arg-D-Trp-L-Cys- NH$_2$	1	18	10
PL-1830	ReO[V]	Heptanoyl-L-Nal 2'-D-Phe(2'-Cl)-L-Arg-D-Trp-L-Cys- NH$_2$	1	5	11
PL-1831	ReO[V]	Heptanoyl-L-Bip-D-Phe(2'-Cl)-L-Arg-D-Trp-L-Cys- NH$_2$	1	-2	13
PL-1832	ReO[V]	Heptanoyl-L-Phe(3,4 Di-Cl)-D-Phe(2'-Cl)-L-Arg-D-Trp-L-Cys- NH$_2$	1	25	12
PL-1833	ReO[V]	Heptanoyl-L-Tle-D-Phe(2'-Cl)-L-Arg-D-Trp-L-Cys- NH$_2$	1	59	49
PL-1834	ReO[V]	Heptanoyl-L-Cha-D-Phe(2'-Cl)-L-Arg-D-Trp-L-Cys- NH$_2$	1	16	46
PL-1835	ReO[V]	Heptanoyl-L-Phe(p-NO$_2$)-D-Phe(2'-Cl)-L-Arg-D-Trp-L-Cys- NH$_2$	1	20	15
PL-1836	ReO[V]	Heptanoyl-L-Hphe-D-Phe(2'-Cl)-L-Arg-D-Trp-L-Cys- NH$_2$	1	82	50
PL-1837	ReO[V]	Heptanoyl-L-Tic-D-Phe(2'-Cl)-L-Arg-D-Trp-L-Cys- NH$_2$	1	42	24
PL-1838	ReO[V]	D-TicL-Arg-L-Trp-L-Cys- NH$_2$	1	54	74
PL-1839	ReO[V]	Ac-D-Tic-D-Phe-L-Arg-L-Trp-L-Cys- NH$_2$	1	11	35
PL-1840	ReO[V]	Ac-L-Tic-D-Phe-L-Arg-L-Trp-L-Cys- NH$_2$	1	34	24
PL-1841	ReO[V]	Ac-L-Pro-L-His-D-Phe-L-Arg-L-Trp-L-Cys- NH$_2$	1	34	61
PL-1842	ReO[V]	Ac-L-Nle-L-Ala-L-His-L-Arg-L-Phe-L-Trp-L-Cys- NH$_2$	1	2	27
PL-1843	ReO[V]	Ac-L-Nle-L-Ala-L-His-D-Arg-L-Phe-L-Trp-L-Cys- NH$_2$	1	-8	16

TABLE 1 Melanoc rtin Rec ptor Scre ning Results: R ceptor Binding Assay				% Inhibition	
Com-pound ID	Metal ion / Linear peptide	Sequence Structure	Conc. Cut off (μM)	MC4-R	MC1-R (B-16)
PL-1844	ReO[V]	Ac-L-Nle-L-Ala-L-His-D-Phe-L-Arg-L-Trp-D-Cys-L-Trp- NH$_2$	1	83	98
PL-1845	ReO[V]	Ac-L-Nle-L-Ala-L-His-D-Phe-L-Arg-L-Trp-L-Cys-D-Trp- NH$_2$	1	96	99
PL-1846	ReO[V]	Ac-L-Nle-L-Ala-L-His-D-Phe-L-Arg-L-Cys-L-Trp- NH$_2$	0.1	4	85
PL-1849	ReO[V]	C$_6$H$_5$-CO-L-Lys-D-Phe-L-Cys-L-Trp- NH$_2$	1	12	39
PL-1850	ReO[V]	C$_6$H$_5$-CH=CH-CO-L-Lys-D-Phe-L-Cys-L-Trp-NH$_2$	1	-2	24
PL-1851	ReO[V]	Pyridine-3-CO-L-Lys-D-Phe-L-Cys-L-Trp- NH$_2$	1	-5	26
PL-1852	ReO[V]	Tetralin-2-CO-L-Lys-D-Phe-L-Cys-L-Trp- NH$_2$	1	0	15
PL-1853	ReO[V]	Naphthalene-1-CO-L-Lys-D-Phe-L-Cys-L-Trp-NH$_2$	1	9	27
PL-1854	ReO[V]	Naphthalene-2-CO-L-Lys-D-Phe-L-Cys-L-Trp-NH$_2$	1	-6	24
PL-1855	ReO[V]	Z-L-Lys-Gly-D-Phe(4'-Cl)-L-Cys-L-Trp- NH$_2$	1	0	32
PL-1856	ReO[V]	Z-L-Lys-L-Val-D-Phe(4'-Cl)-L-Cys-L-Trp- NH$_2$	1	31	53
PL-1857	ReO[V]	Z-L-Lys-L-Nle-D-Phe(4'-Cl)-L-Cys-L-Trp- NH$_2$	1	32	40
PL-1858	ReO[V]	Z-L-Lys-L-Leu-D-Phe(4'-Cl)-L-Cys-L-Trp- NH$_2$	1	31	36
PL-1859	ReO[V]	Ac-L-Phe-L-Phe-L-Cys-L-Tic-L-Lys- NH$_2$	1	-8	9
PL-1860	ReO[V]	Ac-L-Phe-L-Phe-L-Cys-L-Inp-L-Lys- NH$_2$	1	0	6
PL-1861	ReO[V]	Ac-L-Phe-L-Phe-L-Cys-4-Abz-L-Lys- NH$_2$	1	-14	0
PL-1862	ReO[V]	Ac-L-Phe-L-Phe-L-Cys-3-Abz-L-Lys- NH$_2$	1	-7	17
PL-1863	ReO[V]	Ac-L-Phe-L-Phe-L-Cys-2-Abz-L-Lys- NH$_2$	1	6	19
PL-1864	ReO[V]	Ac-L-Phe-D-Trp-L-Cys-2-Abz-L-Lys- NH$_2$	1	-7	17
PL-1865	ReO[V]	Ac-L-Ser(Bzl)-D-Phe(2'-Cl)-L-Arg-D-Trp-L-Cys-NH$_2$	1	40	13
PL-1866	ReO[V]	Bz-L-Ser(Bzl)-D-Phe(2'-Cl)-L-Arg-D-Trp-L-Cys-NH$_2$	1	30	16
PL-1867	ReO[V]	Heptanoyl-L-Asn-D-Phe(2'-Cl)-L-Arg-D-Trp-L-Cys- NH$_2$	1	60	52
PL-1868	ReO[V]	Heptanoyl-L-Asp-D-Phe(2'-Cl)-L-Arg-D-Trp-L-Cys- NH$_2$	1	-3	5
PL-1869	ReO[V]	Heptanoyl-L-Lys(NH-Bz)-D-Phe(2'-Cl)-L-Arg-D-Trp-L-Cys- NH$_2$	1	42	25
PL-1870	ReO[V]	Heptanoyl-D-B-Hphe(4-F)-L-Arg-D-Trp-L-Cys-NH$_2$	1	11	12
PL-1871	ReO[V]	Heptanoyl-D-B-Hphe(2'-Cl)-L-Arg-D-Trp-L-Cys-NH$_2$	1	3	10
PL-1872	ReO[V]	Ac-D-Ala-L-His-L-Cys-D-Phe(2-Cl)-L-Arg-L-Trp- NH$_2$	1	79	27
PL-1873	ReO[V]	Ac-L-Nle-L-Ala-L-His-D-Phe-L-Arg-D-Cys-Trp-NH$_2$	1	31	92.6
PL-1874	ReO[V]	Ac-L-Nle-L-Ala-L-His-D-Phe-L-Arg-L-Trp-D-Cys-D-Trp- NH$_2$	1	90	98
PL-1875	ReO[V]	1-Naphthl ne-ac tyl-L-Lys-L-Ala-D-Ph (4'-Cl)-L-Cys-Trp- NH$_2$	1	77	34
PL-1876	R O[V]	2-Naphthlene-acetyl-L-Lys-L-Ala-D-Ph (4'-Cl)-L-Cys-Trp-NH$_2$	1	52	8
PL-1877	R O[V]	3-Bromoph nyl acetyl-L-Lys-L-Ala-D-Phe(4'-I)-L-Cys-Trp-NH$_2$	1	92	31

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TABLE 1 Melanocortin Recept r Screening R sults: Receptor Binding Assay				% Inhibition	
Com-pound ID	Metal ion / Linear peptide	Sequence Structure	Conc. Cut off (µM)	MC4-R	MC1-R (B-16)
PL-1878	ReO[V]	4-Bromophenyl acetyl-L-Lys-L-Ala-D-Phe(p-I)-L-Cys-Trp- NH$_2$	1	75	53
PL-1879	ReO[V]	Heptanoyl-L-Ser(Bzl)-D-Phe(3'-Cl)-L-Arg-D-Trp-L-Cys-NH$_2$	1	86	28
PL-1880	ReO[V]	Heptanoyl-L-Ser(Bzl)-D-Phe(3,4-Cl$_2$)-L-Arg-D-Trp-L-Cys- NH$_2$	1	96	18
PL-1881	ReO[V]	Heptanoyl-L-Ser(Bzl)-D-HPhe-L-Arg-D-Trp-L-Cys- NH$_2$	1	-6	16
PL-1882	ReO[V]	Heptanoyl-L-Ser(Bzl)-D-Tic-L-Arg-D-Trp-L-Cys- NH$_2$	1	-14	11
PL-1883	ReO[V]	Heptanoyl-L-Ser(Bzl)-D-Phe(4'-Cl)-L-Arg-D-Trp-L-Cys-NH$_2$	1	97	49
PL-1884	ReO[V]	Ac-D-Phe-L-His-L-Cys-D-Phe(2'-Cl)-L-Arg-L-Trp- NH$_2$	1	64	32
PL-1885	ReO[V]	Ac-D-Nle-L-His-L-Cys-D-Phe(2'-Cl)-L-Arg-L-Trp- NH$_2$	1	84	68
PL-1886	ReO[V]	Ac-D-HPhe-L-His-L-Cys-D-Phe(2'-Cl)-L-Arg-L-Trp- NH$_2$	1	73	62
PL-1887	ReO[V]	Ac-D-Phe-L-Phe-L-Cys-D-Phe(2'-Cl)-L-Arg-L-Trp- NH$_2$	1	50	24
PL-1888	ReO[V]	Ac-D-Ala-L-Nle-L-Cys-D-Phe(2'-Cl)-L-Arg-L-Trp- NH$_2$	1	90	40
PL-1889	ReO[V]	Ac-L-Nle-L-His-L-Cys-D-Phe(2'-Cl)-L-Arg-L-Trp- NH$_2$	1	49	58
PL-1890	ReO[V]	Heptanoyl-D-Ala-L-His-L-Cys-D-Phe(2'-Cl)-L-Arg-L-Trp-NH$_2$	1	24	31
PL-1891	ReO[V]	Heptanoyl-L-Ser(Bzl)-D-BHphe(2'-Cl)-L-Arg-D-Trp-L-Cys-NH$_2$	1	-7	10
PL-1892	ReO[V]	Heptanoyl-L-Ser(Bzl)-D-BHphe(2'-F)-L-Arg-D-Trp-L-Cys- NH$_2$	1	6	10
PL-1893	ReO[V]	Ac-D-Phg-L-His-L-Cys-D-Phe(2'-Cl)-L-Arg-L-Trp- NH$_2$	1	78	52
PL-1894	ReO[V]	Ac-D-Ala-L-Phg-L-Cys-D-Phe(2'-Cl)-L-Arg-L-Trp- NH$_2$	1	81	37
PL-1895	ReO[V]	Ac-D-Ala-L-Phe-L-Cys-D-Phe(2'-Cl)-L-Arg-L-Trp- NH$_2$	1	63	19
PL-1896	ReO[V]	Ac-D-Nle-L-His-L-Cys-D-(NMe)Phe-L-Arg-L-Trp- NH$_2$	1	-1	56
PL-1897	ReO[V]	Heptanoyl-L-Ser(Bzl)-D-Tiq-L-Arg-D-Trp-L-Cys- NH$_2$	1	-24	12
PL-1899	ReO[V]	C$_6$H$_5$(CH$_2$)$_3$-CO-L-Ser(Bzl)-D-(NMe)-Phe-L-Arg-D-Trp-L-Cys- NH$_2$	1	0	19
PL-1900	ReO[V]	3-Bromophenyl acetyl-L-Lys-L-Ala-D-Trp-L-Cys-L-Trp-NH$_2$	1	47	10
PL-1901	ReO[V]	3-Bromophenyl acetyl-L-Lys-L-Ala-D-Phe-L-Cys-L-Trp-NH$_2$	1	51	26
PL-1902	ReO[V]	3-Bromophenyl acetyl-L-Lys-L-Ala-D-Ph (4'-Me)-L-Cys-L-Trp-NH$_2$	1	93	17
PL-1903	ReO[V]	3-Bromophenyl acetyl-L-Lys-L-Ala-D-Ph (3'-Cl)-L-Cys-L-Trp- NH$_2$	1	71	5
PL-1904	R O[V]	3-Bromophenyl acetyl-L-Lys-L-Ala-D-HPhe-L-Cys-L-Trp-NH$_2$	1	11	2

| TABLE 1 | | | | % Inhibition | |
| Melanocortin Receptor Screening Results: Receptor Binding Assay | | | | | |
Com-pound ID	Metal ion / Linear peptide	Sequence Structure	Conc. Cut off (µM)	MC4-R	MC1-R (B-16)
PL-1905	ReO[V]	2-Chlorophenyl acetyl-L-Lys-L-Ala-D-Phe(4'-Cl)-L-Cys-L-Trp- NH$_2$	1	86	31
PL-1906	ReO[V]	4-Chlorophenyl acetyl-L-Lys-L-Ala-D-Phe(4'-Cl)-L-Cys-L-Trp- NH$_2$	1	91	68
PL-1907	ReO[V]	4-Methylphenyl acetyl-L-Lys-L-Ala-D-Phe(4'-Cl)-L-Cys-L-Trp- NH$_2$	1	69	44
PL-1908	ReO[V]	Indonyl acetyl-L-Lys-L-Ala-D-Phe(4'-Cl)-L-Cys-L-Trp-NH$_2$	1	33	8
PL-1909	ReO[V]	3-Bromophenyl acetyl-L-Arg-L-Ala-D-Phe(4'-Cl)-L-Cys-L-Trp- NH$_2$	1	95	32
PL-1910	ReO[V]	Heptanoyl-L-Dpr(Bz)-D-Phe(2'-Cl)-L-Arg-D-Trp-L-Cys-NH$_2$	1	23	42
PL-1911	ReO[V]	Heptanoyl-L-Dpr(2'-Naphthlene acetyl)-D-Phe(2'-Cl)-L-Arg-D-Trp-L-Cys-NH$_2$	1	-6	11
PL-1912	ReO[V]	Heptanoyl-L-Dpr(1'-Admantane carbonyl)-D-Phe(2'-Cl)-L-Arg-D-Trp-L-Cys-NH$_2$	1	-3	2
PL-1913	ReO[V]	Heptanoyl-L-Dpr(4'-MePhenyl acetyl)-D-Phe(2'-Cl)-L-Arg-D-Trp-L-Cys-NH$_2$	1	22	35
PL-1914	ReO[V]	Heptanoyl-L-Dpr(3'-BrPhenyl acetyl)-D-Phe(2'-Cl)-L-Arg-D-Trp-L-Cys-NH$_2$	1	20	53
PL-1915	ReO[V]	Heptanoyl-L-Ser(Bzl)-D-Phe(2'-Cl)-L-Arg-L-Trp-L-Cys-NH$_2$	1	94	72
PL-1916	ReO[V]	Heptanoyl-L-Ser(Bzl)-D-Phe(2'-Cl)-L-Arg-L-His-L-Cys-NH$_2$	1	9	44
PL-1917	ReO[V]	Heptanoyl-L-Ser(Bzl)-D-Phe(2'-Cl)-L-Arg-L-Nal 2'-L-Cys-NH$_2$	1	94	48
PL-1918	ReO[V]	Heptanoyl-L-Ser(Bzl)-D-Phe(2'-Cl)-L-Arg-L-Bip-L-Cys-NH$_2$	1	10	21
PL-1919	ReO[V]	Heptanoyl-L-Ser(Bzl)-D-Phe(2'-Cl)-L-Arg-L-Pal 3'-L-Cys-NH$_2$	1	17	47
PL-1920	ReO[V]	D-Phe-L-Arg-L-Trp-L-Cys-NH$_2$	1	52	65
PL-1921	ReO[V]	Ac-D-Phe-L-Arg-L-Trp-L-Cys-NH$_2$	1	20	25
PL-1922	ReO[V]	Ac-L-Nle-D-Phe-L-Arg-L-Trp-L-Cys-NH$_2$	1	25	28
PL-1923	ReO[V]	Ac-L-Nle-L-Ala-D-Phe-L-Arg-L-Trp-L-Cys-NH$_2$	1	68	70
PL-1924	ReO[V]	Ac-L-Pro-D-Phe-L-Arg-L-Trp-L-Cys-NH$_2$	1	44	33
PL-1925	ReO[V]	Heptanoyl-D-Phe-L-Arg-L-Trp-L-Cys-NH$_2$	1	6	18
PL-1926	ReO[V]	Bz-L-Arg-L-Trp-L-Cys- NH$_2$	1	7	25
PL-1927	ReO[V]	Phenyl acetyl-L-Arg-L-Trp-L-Cys- NH$_2$	1	8	28
PL-1928	ReO[V]	3-Phenyl-propanoyl-L-Arg-L-Trp-L-Cys- NH$_2$	1	8	32
PL-1929	ReO[V]	4-Phenyl-butanoyl-L-Arg-L-Trp-L-Cys- NH$_2$	1	2	18
PL-1930	ReO[V]	t-Cinnamoyl-L-Arg-L-Trp-L-Cys- NH$_2$	1	-20	9
PL-1931	ReO[V]	1-Naphthyl-acetyl-L-Arg-L-Trp-L-Cys- NH$_2$	10	92	47
PL-1932	ReO[V]	2-Naphthyl-acetyl-L-Arg-L-Trp-L-Cys- NH$_2$	1	1	16
PL-1933	ReO[V]	1-Naphthoyl-L-Arg-L-Trp-L-Cys- NH$_2$	1	0	14
PL-1934	ReO[V]	2-Naphthoyl-L-Arg-L-Trp-L-Cys- NH$_2$	1	6	34
PL-1935	ReO[V]	Heptanoyl-L-Arg-L-Trp-L-Cys-NH$_2$	1	8	39
PL-1936	ReO[V]	Heptanoyl-L-Ser(Bzl)-D-Phe(4'-F)-L-Arg-L-Trp-L-Cys-NH$_2$	1	81	71
PL-1937	ReO[V]	Heptanoyl-L-Ser(Bzl)-D-Phe(penta-F)-L-Arg-L-Trp-L-Cys- NH$_2$	1	91	65
PL-1938	ReO[V]	Heptanoyl-L-Ser(Bzl)-D-Pal (2)-L-Arg-L-Trp-L-Cys- NH$_2$	1	16	16

Com-pound ID	Metal ion / Linear peptide	Sequence Structure	Conc. Cut off (μM)	MC4-R	MC1-R (B-16)
TABLE 1 — Melanocortin Receptor Screening Results: Receptor Binding Assay — % Inhibition					
PL-1939	ReO[V]	Heptanoyl-L-Ser(Bzl)-D-Phe(2'-Br)-L-Arg-L-Trp-L-Cys- NH_2	1	91	73
PL-1940	ReO[V]	Ac-D-Ala-L-Nle-L-Cys-D-Phe (2'-Cl)-L-Arg-L-Trp- NH_2	1	89	26
PL-1941	ReO[V]	Heptanoyl-L-Ser(Bzl)-D-Phe(2'-Cl)-L-Lys-L-Nal 2'-L-Cys- NH_2	1	90	25
PL-1942	ReO[V]	Heptanoyl-L-Ser(Bzl)-D-Phe(2'-Cl)-L-Arg-L-Nal 1'-L-Cys- NH_2	1	53	16
PL-1943	ReO[V]	Heptanoyl-L-Ser(Bzl)-D-Phe(penta-F)-L-Arg-L-Nal 2'-L-Cys-NH_2	1	93	64
PL-1944	ReO[V]	Heptanoyl-L-Ser(Bzl)-D-Phe(penta-F)-L-Lys-L-Nal 2'-L-Cys-NH_2	1	80	40
PL-1945	ReO[V]	Heptanoyl-L-Hyp(Bzl)-D-Phe(2-Cl)-L-Arg-L-Nal 2'-L-Cys-NH_2	1	94	30
PL-1946	ReO[V]	Heptanoyl-[(2S,3R),5-phenyl pyrrolidinyl-2-carbonyl]-D-Phe(2-Cl)-L-Arg-L-Nal 2'-L-Cys-NH_2	1	81	24
PL-1947	ReO[V]	Heptanoyl-L-Ser(Bzl)-D-Phe(4'-CF3)-L-Arg-L-Trp-L-Cys-NH_2	1	98	23
PL-1948	ReO[V]	Heptanoyl-L-Ser(Bzl)-D-Phe(4'-CH3)-L-Arg-L-Trp-L-Cys-NH_2	1	98	50
PL-1949	ReO[V]	Heptanoyl-L-Ser(Bzl)-D-Phe(4'-Cl)-L-Arg-L-Trp-L-Cys-NH_2	1	99	77
PL-1950	ReO[V]	Heptanoyl-L-Ser(Bzl)-D-Phe(3'-Cl)-L-Arg-L-Trp-L-Cys-NH_2	1	92	37
PL-1951	ReO[V]	Ac-D-Val-L-His-L-Cys-D-Phe(2' Cl)-L-Arg-L-Trp-NH_2	1	60	32
PL-1952	ReO[V]	Ac-D-Ala-L-Chg-L-Cys-D-Phe(2' Cl)-L-Arg-L-Trp-NH_2	1	88	46
PL--1953	ReO[V]	Ac-D-Ala-L-Val-L-Cys-D-Phe(2' Cl)-L-Arg-L-Trp-NH_2	1	69	19
PL-1955	ReO[V]	Ac-D-Chg-L-His-L-Cys-D-Phe(2' Cl)-L-Arg-L-Trp-NH_2	1	76	68
PL-1956	ReO[V]	Ac-D-Cha-L-His-L-Cys-D-Phe(2' Cl)-L-Arg-L-Trp-NH_2	1	58	64
PL-1957	ReO[V]	Ac-D-Leu-L-His-L-Cys-D-Phe(2' Cl)-L-Arg-L-Trp-NH_2	1	66	53
PL-1958	ReO[V]	Ac-D-Val-L-Phg-L-Cys-D-Phe(2' Cl)-L-Arg-L-Trp-NH_2	1	60	31
PL-1959	ReO[V]	Ac-D-Chg-L-Phg-L-Cys-D-Phe(2' Cl)-L-Arg-L-Trp-NH_2	1	55	27
PL-1960	ReO[V]	Ac-D-Cha-L-Phg-L-Cys-D-Phe(2' Cl)-L-Arg-L-Trp-NH_2	1	38	16
PL-1961	ReO[V]	Ac-D-Leu-L-Phg-L-Cys-D-Phe(2' Cl)-L-Arg-L-Trp-NH_2	1	77	38
PL-1962	R O[V]	Ac-D-Val-L-Phe-L-Cys-D-Phe(2' Cl)-L-Arg-L-Trp-NH_2	1	47	23
PL-1963	ReO[V]	Ac-D-Chg-L-Phe-L-Cys-D-Phe(2' Cl)-L-Arg-L-Trp-NH_2	1	31	27
PL-1964	R O[V]	Ac-D-Cha-L-Phe-L-Cys-D-Ph (2' Cl)-L-Arg-L-Trp-NH_2	1	28	38

TABLE 1 Melanocortin Receptor Screening Results: Receptor Binding Assay				% Inhibition	
Com-pound ID	Metal ion / Linear peptide	Sequence Structure	Conc. Cut off (µM)	MC4-R	MC1-R (B-16)
PL-1965	ReO[V]	Ac-D-Leu-L-Phe-L-Cys-D-Phe(2' Cl)-L-Arg-L-Trp-NH$_2$	1	62	46
PL-1970	ReO[V]	Phenylacetyl-L-Arg-L-Ala-D-Phe(4'-Cl)-L-Cys-D-Trp-NH$_2$	1	92	51
PL-1971	ReO[V]	3'-bromophenylacetyl-L-Arg-D-Ala-Dphe(4'-Cl)-L-Cys-L-Trp-NH$_2$	1	33	67
PL-1972	ReO[V]	Phenylacetyl-D-Arg-L-Ala-D-Phe(4'-Cl)-L-Cys-L-Trp-NH$_2$	1	17	27

        For selected peptides of this invention, a cAMP assay was also performed. Human MC4-R or
B-16 cells were grown to confluence in 96 well plates (plating approximately 250,000 cells per well).
Identical sets of cells in triplicate were treated with 0.2 mM isobutylmethylxanthine (IBMX) and the
5   chosen concentration of the rhenium metal ion-complexed peptide and the rhenium metal ion-
complexed peptide in the presence of 20 nM alpha-MSH. Cells similarly treated but with only 20 nM
alpha-MSH served as positive control. A buffer blank, as a negative control, was also included.
Incubation was for one hour at 37°C after which the medium was aspirated and the cells extarted with
150 microliters of HCl. Total cAMP accumulated in 100 microliters of this solution was quantitated
10   using a commercially available low pH cAMP assay kit (R&D Systems) by the procedure specified by
the kit supplier. The table shows the amount of cAMP accumulated in the cells upon exposure to the
test compound alone and the presence of 20 nM alpha-MSH. The rhenium metal ion-complexed
peptide showing cAMP accumulation in the same range as or higher than the positive control (buffer
blank in the presence of alpha-MSH) are considered to be agonist ligands. The rhenium metal ion-
15   complexed peptide showing accumulation in the same range as the negative control (buffer blank in
the absence of alpha-MSH) are ineffective at the test concentration if the result is similar to the positive
control where alpha-MSH is also present in the assay. The rhenium metal ion-complexed peptide
showing accumulation in the same range as the negative control are considered to be antagonists if
there is inhibition in cAMP when alpha-MSH is present in the assay. Values above the positive control
20   are due to potent agonism, experimental variance or synergistic agonistic effects of rhenium metal ion-
complexed peptide and alpha-MSH.

Table 2 Evaluation of MC-specific metallopeptides of the primary structure of **Table 1** in cAMP stimulation assay in presence and absence of natural stimulator α-MSH				
ID	METAL ION	cAMP Accumulation (pMol/ml)		
		µM Concentration	Absence of α-MSH	Presence of α-MSH
Buffer blank			1	99
PL-815	ReO[V]	10	55	178
PL-836	ReO[V]	10	0.31	68
PL-837	ReO[V]	10	9	63
PL-838	ReO[V]	10	2	53
PL-1102	ReO[V]	10	2	113

Table 2				
Evaluation of MC-specific m tallopeptides of the primary structur of **Table 1** in cAMP stimulation assay in presence and absence of natural stimulator α-MSH				
ID	METAL ION	cAMP Accumulation (pMol/ml)		
		µM Concentration	Absence of α-MSH	Presence of α-MSH
PL-1113	ReO[V]	10	123	67
PL-1145	ReO[V]	10	221	277
PL-1160	ReO[V]	10	18.	34
PL-1165	ReO[V]	10	15	71
PL-1179	ReO[V]	10	7	50
PL-1183	ReO[V]	1	42	39
PL-1185	ReO[V]	1	30	22
PL-1189	ReO[V]	1	52	34
PL-1190	ReO[V]	1	63	148
PL-1191	ReO[V]	1	8	94
PL-1207	ReO[V]	1	4	3
PL-1208	ReO[V]	10	2	2
PL-1209	ReO[V]	1	5	13
PL-1210	ReO[V]	1	8	5
PL-1211	ReO[V]	1	10	8
PL-1222	ReO[V]	50	177	346
PL-1223	ReO[V]	10	63	85
PL-1233	ReO[V]	10	19	21
PL-1235	ReO[V]	10	29	28
PL-1236	ReO[V]	10	11	30
PL-1237	ReO[V]	10	96	116
PL-1238	ReO[V]	10	26	140
PL-1239	ReO[V]	10	108	131
PL-1249	ReO[V]	10	4	139
PL-1250	ReO[V]	10	2	15
PL-1251	ReO[V]	10	7	21
PL-1255	ReO[V]	10	2	2
PL-1256	ReO[V]	1	17	71
PL-1258	ReO[V]	10	9	63
PL-1259	ReO[V]	1	131	131
PL-1260	ReO[V]	1	5	6
PL-1261	ReO[V]	1	189	214
PL-1264	ReO[V]	1	1	11
PL-1265	ReO[V]	1	3	127
PL-1266	ReO[V]	1	2	131
PL-1268	ReO[V]	1	18	16
PL-1269	ReO[V]	1	4	2
PL-1270	None	1	139	123
PL-1272	None	1	80	75
PL-1276	ReO[V]	1	3	12
PL-1281	ReO[V]	1	158	25
PL-1285	ReO[V]	1	148	158
PL-1292	ReO[V]	1	3	13
PL-1293	ReO[V]	1	3	6
PL-1294	ReO[V]	1	43	91
PL-1295	R O[V]	1	136	122
PL-1298	ReO[V]	1	1	32
PL-1297	ReO[V]	1	3	7
PL-1301	ReO[V]	1	1	23
PL-1302	ReO[V]	1	12	43
PL-1303	ReO[V]	1	2	59

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Table 2				
Evaluation of MC-specific metallop ptides of the primary structur of **Tabl 1** in cAMP stimulation assay in presence and absence of natural stimulator α-MSH				
ID	METAL ION	cAMP Accumulation (pMol/ml)		
		μM Concentration	Absence of α-MSH	Presence of α-MSH
PL-1305	ReO[V]	1	2	3
PL-1306	ReO[V]	1	189	158
PL-1329	ReO[V]	1	1	63
PL-1332	ReO[V]	1	0	103
PL-1335	ReO[V]	1	0	3
PL-1340	ReO[V]	1	3	7
PL-1342	ReO[V]	10	43	59
PL-1346	ReO[V]	10	3	76
PL-1347	ReO[V]	10	2	81
PL-1351	ReO[V]	10	68	.30
PL-1370	ReO[V]	1	2	16
PL-1372	ReO[V]	1	13	149
PL-1373	ReO[V]	1	1	8
PL-1374	ReO[V]	1	37	73
PL-1387	None	1	52	94
PL-1396	ReO[V]	1	36	120
PL-1438	ReO[V]	1	113	126
PL-1440	ReO[V]	1	59	55
PL-1443	ReO[V]	1	4	34
PL-1451	ReO[V]	1	9	90
PL-1493	ReO[V]	1	4	15
PL-1581	ReO[V]	1	111	132
PL-1658	ReO[V]	1	10	67
PL-1659	ReO[V]	1	7	78
PL-1662	ReO[V]	1	6	96
PL-1663	ReO[V]	1	55	122
PL-1664	ReO[V]	1	7	44
PL-1665	ReO[V]	1	10	70
PL-1666	ReO[V]	1	15	28
PL-1667	ReO[V]	1	23	63
PL-1684	ReO[V]	1	5	110
PL-1685	ReO[V]	1	7	102
PL-1686	ReO[V]	1	4	106
PL-1690	ReO[V]	1	13	76
PL-1691	None	1	30	102
PL-1692	None	1	18	106
PL-1694	ReO[V]	1	25	92
PL-1695	ReO[V]	1	30	101
PL-1702	ReO[V]	1	4	134
PL-1703	ReO[V]	1	2	73
PL-1704	ReO[V]	1	3	73
PL-1705	ReO[V]	1	2	74
PL-1706	ReO[V]	1	2	71
PL-1707	ReO[V]	1	15	156
PL-1708	ReO[V]	1	13	54
PL-1709	R O[V]	1	33	46
PL-1710	R O[V]	1	3	7
PL-1718	ReO[V]	1	23	105
PL-1722	R O[V]	1	28	180
PL-1723	R O[V]	1	30	169
PL-1726	ReO[V]	1	21	171

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Tabl 2 Evaluation of MC-specific m tallopeptid s of th primary structur of **Table 1** in cAMP stimulation assay in presence and absence of natural stimulator α-MSH				
ID	METAL ION	cAMP Accumulation (pMol/ml)		
		µM Concentration	Absence of α-MSH	Presence of α-MSH
PL-1727	ReO[V]	1	27	151
PL-1728	ReO[V]	1	15	164
PL-1730	ReO[V]	1	22	111
PL-1731	ReO[V]	1	19	117
PL-1732	ReO[V]	1	14	105
PL-1733	ReO[V]	1	16	114
PL-1734	ReO[V]	1	13	122
PL-1735	ReO[V]	1	20	115
PL-1736	ReO[V]	1	17	152
PL-1737	ReO[V]	1	53	162
PL-1738	ReO[V]	1	7	173
PL-1751	ReO[V]	1	71	96
PL-1752	ReO[V]	1	4	101
PL-1753	ReO[V]	1	39	104
PL-1754	ReO[V]	1	3	100
PL-1755	ReO[V]	1	21	110
PL-1756	ReO[V]	1	43	102
PL-1757	ReO[V]	1	11	96
PL-1758	ReO[V]	1	34	61
PL-1759	ReO[V]	1	24	79
PL-1760	ReO[V]	1	66	80
PL-1761	ReO[V]	1	11	87
PL-1762	ReO[V]	1	49	88
PL-1774	ReO[V]	1	48	163
PL-1775	ReO[V]	1	53	170
PL-1776	ReO[V]	1	26	138
PL-1778	ReO[V]	1	50	141
PL-1779	ReO[V]	1	47	155
PL-1780	ReO[V]	1	8	126
PL-1782	ReO[V]	1	7	108
PL-1788	ReO[V]	1	189	109
PL-1789	ReO[V]	1	96	90
PL-1790	ReO[V]	1	164	149
PL-1791	ReO[V]	1	163	137
PL-1792	ReO[V]	1	187	109
PL-1793	ReO[V]	1	119	98
PL-1794	ReO[V]	1	4	133
PL-1795	ReO[V]	1	71	124
PL-1796	ReO[V]	1	4	133
PL-1799	ReO[V]	1	9	117
PL-1801	ReO[V]	1	42	107
PL-1805	ReO[V]	1	15	78
PL-1806	ReO[V]	1	10	110
PL-1807	ReO[V]	1	103	115
PL-1808	R O[V]	1	109	137
PL-1809	ReO[V]	1	113	130
PL-1810	ReO[V]	1	101	125
PL-1811	ReO[V]	1	117	66
PL-1812	ReO[V]	1	132	93
PL-1813	ReO[V]	1	36	91
PL-1814	ReO[V]	1	8	80

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Tabl 2
Evaluation of MC-specific m tallopeptid s of the primary structure of **Tabl 1** in cAMP stimulation assay in presence and absence of natural stimulator α-MSH

ID	METAL ION	cAMP Accumulation (pMol/ml)		
		µM Concentration	Absence of α-MSH	Presence of α-MSH
PL-1815	ReO[V]	1	34	119
PL-1816	ReO[V]	1	69	89
PL-1817	ReO[V]	1	24	110
PL-1818	ReO[V]	1	6	96
PL-1819	ReO[V]	1	93	96
PL-1820	ReO[V]	1	8	102
PL-1821	ReO[V]	1	37	88
PL-1822	ReO[V]	1	13	101
PL-1823	ReO[V]	1	14	114
PL-1824	ReO[V]	1	71	124
PL-1825	ReO[V]	1	4	133
PL-1825	ReO[V]	1	7	106
PL-1838	ReO[V]	1	16	65
PL-1839	ReO[V]	1	63	77
PL-1840	ReO[V]	1	76	77
PL-1841	ReO[V]	1	91	81
PL-1842	ReO[V]	1	25	76
PL-1843	ReO[V]	1	18	81
PL-1844	ReO[V]	1	110	81
PL-1845	ReO[V]	1	82	38
PL-1877	ReO[V]	1	72	125
PL-1879	ReO[V]	1	75	108
PL-1880	ReO[V]	1	16	92
PL-1883	ReO[V]	1	125	115
PL-1884	ReO[V]	1	108	158
PL-1885	ReO[V]	1	135	148
PL-1886	ReO[V]	1	151	161
PL-1900	ReO[V]	1	19	87
PL-1902	ReO[V]	1	56	143
PL-1903	ReO[V]	1	62	108
PL-1905	ReO[V]	1	59	96
PL-1906	ReO[V]	1	68	87
PL-1907	ReO[V]	1	62	98
PL-1909	ReO[V]	1	70	81
PL-1915	ReO[V]	1	123	128
PL-1917	ReO[V]	1	109	116
PL-1931	ReO[V]	10	15	79
PL-1144	ReO[V]	10	215	280
PL-1489	ReO[V]	1	9	16
PL-1605	ReO[V]	1	16	15

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Based on the foregoing studies, potential lead molecul s have been identified, including the following:

	PL-1145	MC1-R agonist	(Template 2)
	PL-1144	MC1-R agonist	(Template 2)
5	PL-1493	MC4-R antagonist	(Template 1)
	PL-1883	MC4-R agonist	(Template 1)
	PL-1489	MC4-R antagonist	(Template 5)
	PL-1950	MC4-R specific	(Template 5)
	PL-1947	MC4-R specific	(Template 5)
10	PL-1581	Non-specific agonist	(Template 5)
	PL-1790	Non-specific agonist	(Template 5)
	PL-1791	Non-specific agonist	(Template 5)
	PL-1792	Non-specific agonist	(Template 5)
	PL-1605	Non-specific antagonist	(Template 6)
15	PL-1758	Non-specific	(Template 4)
	PL-1877	MC4-R specific	(Template 4)
	PL-1902	MC4-R specific	(Template 4)
	PL-1909	MC4-R specific agonist	(Template 4)
	PL-1888	MC4-R specific	(Template 7)
20	PL-1269	MC4-R specific antagonist	(Template 3)
	PL-1297	MC4-R specific antagonist	(Template 3)

Radiopharmaceutical Applications. In one embodiment, metallopeptides of this invention that are MC1-R specific can be used, when complexed to ^{99m}Tc as a radiodiagnostic agent, for imaging

25   melanoma tumor metastases, and when complexed to rhenium-188 (^{188}Re), rhenium-186 (^{186}Re) or other therapeutic radionuclides as a radiotherapeutic agent for treatment of melanoma tumors and metastatic tumors.

Human melanoma has a complex antigenic profile. It is generally believed that malignant melanoma is derived by UV activity from DOPA positive melanocytes, the melanin (skin pigment)

30   producing units. Primary diagnosis involves electron microscopic examination to reveal the presence or absence of pre-melanosomes. Melanotic melanoma is classified as dendritic, spindle, bizarre, large epitheloid, small nevus and so on. Amelanotic melanoma, on the other hand, is frequently misdiagnosed because the histology of these cells resembles that of malignant lymphoma, carcinoma or sarcoma. Therefore, morphological evaluation may not prove reliable for clinical diagnosis. Thus,

35   there is a need for a receptor-specific diagnostic test, particularly to identify and locate metastatic m lanoma tumors.

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Sev ral studies hav documented the pr senc of melanotropin recept rs on primary human melanoma cells. Melanotropin receptors hav be n report d as mark rs for melanotic and amelanotic human melanoma tumors. In particular, the presence of MC1-R has been demonstrated in human melanoma cells by an antibody to MC1-R.

5   The melanotropin bioactive sequence, or message segment, is a tetrapeptide, His-Phe-Arg-Trp, that exist as a reverse turn. Within the reverse turn, the His, Phe, and Trp residues have been postulated to form a hydrophobic receptor binding surface. The His residue has recently been identified as a signal residue that helps discriminate between MC1-R and MC4-R. Thus, it is possible to design metallopeptides of this invention which are specific for MC1-R, and bind MC1-R with high

10   affinity, but which are not specific for MC4-R, or which bind MC4-R with low affinity. One result is a ^{99m}Tc-labeled radioimaging agent with high specific affinity and selectivity for the MC1-R. In additi n, both agonists and antagonists metallopeptides of this invention are contemplated for comparative evaluation in imaging melanoma tumors.

The product can be formulated as a single-vial, lyophilized radiolabeling kit containing the

15   peptide in an uncomplexed state, buffer, and a reducing agent for pertechnetate. To induce radiolabeling, resulting in a metallopeptide, the vial is incubated after the addition of sodium pertechnetate.

In one method of labeling with ^{99m}Tc, a 5-10 μg sample of the peptide taken in 0.001 N aq. HCl is mixed with 1-30 mCi of generator-eluted Na^{99m}TcO$_4$ in a 5 ml serum vial. The volume of the

20   resulting mixture is adjusted to 600 μl using injectable saline. A 400 μl volume of freshly prepared and nitrogen-purged phthalate-tartrate-Sn(II) buffer (40:10:1 mM) is added to the vial under a nitrogen head space. The vial is immediately sealed and placed in a shielded boiling water bath. After 15 minutes the vial is removed from the water bath and allowed to come to room temperature. The radiochemical purity, as calculated from HPLC profiles, ranges from 90-99%.

25   The peptides of this invention may alternatively be labeled with ^{99m}Tc by other means, including use of stannous-tartrate-succinate buffer, stannous-EDTA-succinate buffer, stannous stabilized in glucoheptonate, or a stannous-borate-tartrate buffer, as well as other means of labeling with ^{99m}Tc known in the art.

For radiopharmaceutical and other medical applications, the metallopeptides of this inventi n

30   may be delivered to a subject by any means known in the art. This includes intravenous injection, subcutaneous injection, administration through mucous membranes, oral administration, dermal administration, regional administration to an organ, cavity or region, and the like.

Imaging may be any means known in the art, including gamma camera and SPECT imaging. Imaging may comm nce immediately aft r administration, and may include time course radiographic

35   studi s, and imaging may continue so long as images may be obtain d.

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The MC1-R specific metallopeptides of this invention may b used as melan ma specific tumor imaging and staging agent. These uses include arly detection and localization f primary and disseminated lesions, identification of lymph nodes containing lesions, radioimmunoguided surgery applications and the like. Tumor imaging using a ^{99m}Tc-labeled metallopeptide of this invention

5   selective for the MC1-R will further help in formulating the optimal clinical treatment modality, wheth r surgical, radiation or chemotherapeutic.

Chemoprevention Applications. In another embodiment, metallopeptides of this invention that are MC1-R specific can be used as chemoprevention agents against sun-induced, such as by UV radiation, neoplastic activity in human skin. MC1-R agonist metallopeptides of this invention may b

10   employed to stimulate epidermal melanocytes to produce melanin as well as to convert pheomelanin to eumelanin. Eumelanin, which is dark brown or black pigmentation, is considered more photo-protective than pheomelanin, which is yellow or red pigmentation.

In general, darker skinned individuals have lower incidences of skin cancer than lighter skinned people. The dark pigment eumelanin, a brown/black pigment incorporating dopa-based structural

15   units, is the main photoprotective agent in skin. Lighter colored people have higher levels of pheomelanin, a red/yellow pigment having predominantly cysteine and related sulfur-based structural units, which is an inefficient UV absorber. The process of melanogenesis is believed to involve stimulation of MC1-R in epidermal melanocytes, thereby mediating the stimulation of tyrosinase enzymes within these pigment cells, inducing the conversion of tyrosine to dopa and then through

20   dopaquinone to eumelanin. Sun tanning due to direct sun exposure is also proposed to result from the same pathway by local production of melanotropic peptide from a POMC gene in the epidermis. Thus stimulation of eumelanin production and conversion of pheomelanin to eumelanin may be a desirable chemoprevention modality in blocking sun, or UV, induced neoplastic activity in skin.

A potent, high-affinity and highly selective MC1-R agonist metallopeptide of this invention can

25   accordingly be used as a therapeutic chemoprevention agent for combating harmful sun, or UV, exposure that induces neoplastic activity in skin melanocytes.

Particularly for individuals previously diagnosed with melanoma or diagnosed as highly susceptible to melanoma, avoidance of any significant UV exposure is medically necessary. Currently, both physical screens, such as hats, long sleeves and so on, and various sun- or UV-protecting

30   creams and formulations are utilized. However, the efficacy, both absolute and as a function of tim , of even the best chemical sun block is limited, and physical screens are inappropriate for many activities. This results in individuals either receiving unacceptably high UV doses or foregoing normal activities, such as swimming, hiking, skiing, attending sporting events, employment in outdoor settings and so on.

35   Eumelanin production through activation of the MC1-R on pidermal melanocytes provides a natural shield against sun (UV) induced mutations and DNA damag than does any chemical sun block. Eumelanin is a better UV absorber over a wid range than any commercially availabl sun-protecting cream or formulation.

SUBSTITUTE SHEET (RULE 26)

The metallopeptides for this application may be made with non-radioactiv is top s of rh nium, or other metals as specified h rein, with metal ion compl xation by any means specified her in r known in the art. The m tallopeptides may be formulated by any means known in th art, including but not limited to tablets, capsules, caplets, suspensions, powders, lyophilized forms and aerosols and

5   may be mixed and formulated with buffers, binders, stabilizers, anti-oxidants and other agents known in the art. The metallopeptides may be administered by any systemic or partially systemic means known in the art, including but not limited to intravenous injection, subcutaneous injection, administration through mucous membranes, oral administration, dermal administration, skin patches, aerosols and the like.

10   *Therapeutic Applications.* In another embodiment, metallopeptides of this invention that are MC4-R agonists can be used as a therapeutic agent to modify energy metabolism and feeding behavior, including treatment of pathologic obesity and related conditions. Metallopeptides of this invention that are MC4-R antagonists can also be used as a therapeutic agent in eating disorders, such as treatment of anorexia.

15   Control centers for eating and satiety reside in the hypothalamus. These responses are determined by diverse hormones and soluble factors that signal through specific receptors in the hypothalamus. MC4-R is known to be expressed in the brain, and inactivation of this receptor by g n targeting has resulted in mice with the maturity-onset obesity syndrome that is associated with hyperphagia, hyperinsulinemia and hyperglycemia.

20   In yet another embodiment, metallopeptides of this invention may used as therapeutic agents for treatment of sexual dysfunction, including treatment of both male erectile dysfunction and female sexual dysfunction. In yet another embodiment, metallopeptides of this invention may be used as therapeutic agents for treatment of inflammation, including specifically MC1-R and MC3-R agonist metallopeptides.

25   The metallopeptides for this application may be made with non-radioactive isotopes of rhenium, or other metals as specified herein, with metal ion complexation by any means specified herein or known in the art. The metallopeptides may be formulated by any means known in the art, including but not limited to tablets, capsules, caplets, suspensions, powders, lyophilized forms and aerosols and may be mixed and formulated with buffers, binders, stabilizers, anti-oxidants and other agents known

30   in the art. The metallopeptides may be administered by any systemic or partially systemic means known in the art, including but not limited to intravenous injection, subcutaneous injection, administration through mucous membranes, oral administration, dermal administration, skin patches, aerosols and the like.

The invention is further illustrated by the following non-limiting examples.

35   Exampl 1   DEVELOPMENT OF A PROTOTYPE METALLOPEPTIDE LIBRARY FOR THE MELANOCORTIN RECEPTOR.

The library design was based on the tetrapeptide m ssage sequence, His-Phe-Arg-Trp (6-9

s qu nce), of α-MSH. This sequ nce exists as a r v rs turn, making it suitabl for conv rsion into a metallop ptid format of this inv ntion. In this approach metallopeptides were designed around a trip ptide N_3S_1 MBD designed for a rh nium metal ion. The MBD was derivatized to yield th pentapeptide Ac-His-Phe-Arg-Cys-Trp-NH₂ as a putative candidate for melanocortin ("MC") receptors. Further refinements in the structure were made in response to other considerations, including the chirality of amino acid side chains, yielding a template structure Ac-His-D-Phe-Arg-Cys-Trp-NH₂. Th structure of this peptide after binding to rhenium is:
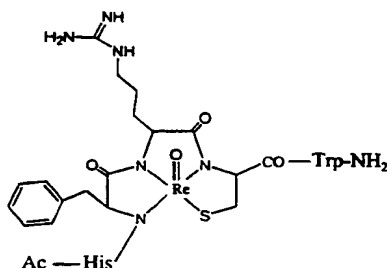
The template structure was used to define a small combinatorial library utilizing split synthesis methodologies. The final template selected for the combinatorial library was Ac-D-His-Xaa-D-Cys-Trp-NH₂, where Xaa was D-(2') Naphthylalanine, D-Trp, D-HomoPhe, or D-Phenylglycine. For this library, the peptide resin, Cys(StBu)-Trp(Boc)-Resin was split in four equal parts. Each part was reacted with one of the four Xaa types. After coupling, the resin pools were mixed and synthesis continued in a single pool to couple the His residue. The final result was four separate peptides in a single pool, each peptide varying by one amino acid, in the Xaa position.

An StBu OSPG group was used to protect the SH group during synthesis. After solid-phase assembly of the peptide chain using Fmoc chemistry with acid labile side chain protecting groups, the StBu group was split using tributylphosphine. The resulting free SH-containing peptide-resin was treated with the rhenium transfer agent $Re(O)Cl_3(PPh_3)_2$ in the presence of 1,8-Diazabicyclo[5,4,0] undec-7-ene as base. The resulting metallopeptide resin was then treated with TFA to cleave it from the resin and de-protect all the side chain protecting groups. The products were analyzed by mass spectrometry. HPLC analysis was performed and individual peaks collected and subjected to mass analysis. The resulting peptides were analyzed by electron spray mass spectrometry, yielding the predicted mass, including the rhenium complexed to the peptide.

Example 2   DESIGN AND SYNTHESIS OF MELANOCORTIN RECEPTOR-SPECIFIC
            METALLOPEPTIDE LIBRARY

The library was rationally designed based upon data relating to melanocortin receptors and peptide sequ nces specific to the m lanocortin receptors, including melanotropin side-chain pharmacophor s, D-Phe[7] and Trp[9], that interact with a hydrophobic n twork of r ceptor aromatic residues in transmembrane r gions 4, 5, 6, and 7. Based on this design crit rions, a pharmacophor for the melanocortin receptor was preliminarily defined, and a combinatorial library designed for

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identification of potent and receptor-selective agonists.

Based on the design crit ria, the putative structur R-Aaa-Baa-L-Cys-Caa-NH$_2$ was selected, in which each of Aaa, Baa and Caa are selected from L- or D-isomers of 2-Nal (1), Phe (2), Trp (3), Tyr (4) and Ala (5), so that any one of the foregoing can be substituted for any one of Aaa, Baa or Caa. In

5   the nomenclature adopted for the library design, the five amino acids were designated 1 through 5, with the isomerism conventionally notated, so that, for example, Baa$_2$L refers to L-Phe in the Baa position.

The terminal R group was selected from Ac, C$_6$H$_5$OOH, CH$_3$(CH$_2$)$_5$-COOH, C$_6$H$_5$CH=CH-COOH (trans) and Pyridine-3-carboxylate. The terminal R group represents a truncated amino acid, and

10   offers additional structural diversity.

A pool and split library synthesis scheme was employed such that 5,000 separate compounds were synthesized, resulting in 200 final pools each containing 25 different compounds, with the compounds differing solely by the amino acids in the Aaa and Baa position. Using this methodology, binding characteristics relating to the Caa amino acid or R terminal group can be identified through

15   inter-group comparison, thereby simplifying the deconvolution strategy.

The library synthesis steps are set forth in **Fig. 8**. The resin of step 1 was divided into 10 groups. At step 2 each of Caa$_1$L through Caa$_5$D were coupled to an individual resin group, and L-Cys was coupled to each resin group, resulting in 10 groups and 20 couplings. Each of the resin groups of step 2 was then divided into 10 sub-groups as shown at step 3 (with only one subgroup illustrated at

20   step 3, and for each subgroup of step 3, each of Baa$_1$L through Baa$_5$D were coupled to one group within the subgroup, resulting in 100 groups in 10 subgroups and 100 couplings. For each subgroup of step 3, the five Baa$_x$L members and the five Baa$_x$D members were separately pooled in step 4, resulting in 20 subgroups, with each subgroup containing five different sequences differing by the Baa$_x$ member. Each of the 20 subgroups of step 4 were then in step 5 divided into 10 groups (with only one

25   shown for illustration purposes in **Fig. 8**), and for each subgroup, each of Aaa$_1$L through Aaa$_5$D were coupled to one group within the subgroup, resulting in 200 groups in 20 subgroups and 200 couplings. For each subgroup of step 5, the five Aaa$_x$L members and the five Aaa$_x$D members were separately pooled in step 6, resulting in 40 subgroups, with each subgroup containing twenty-five different sequences differing by the Baa$_x$ and Aaa$_x$ member. In step 7, each of the 40 subgroups of step 6

30   were divided into five groups, and each of R$_1$ through R$_5$ were coupled to one group within the subgroup, resulting in 200 groups in 40 subgroups, with each group containing 25 different sequences differing by the Baa$_x$ and Aaa$_x$ member.

Peptides were synthesized using Fmoc chemistry, with side chain functionalities protected using acid labile groups. The SH group of the Cys r sidue was protected by a S^{t}Bu OSPG cleavable in

35   presence of both base and acid labile groups using tributylphosphine as the reducing agent. The peptide chain was assembled on th solid phase using 1-(1H-benzotriazole-1-yl)-1,1,3,3,-tetra-methyluronium tetrafluoroborate (TBTU) as a coupling agent. Th SH group was th n selectiv ly unprot cted and rh nium metal ion compl x d using th rh nium transfer agent Re(O)Cl$_3$(PPh$_3$)$_2$ in

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th pr sence of 1,8-Diazabicyclo[5,4,0]und c-7-ene (DBU) as base. In this manner, the m tal-peptid complex was formed with th peptide chain still t thered to the solid support. The metall peptide was then liberated from the solid support by treatment with TFA. This solid phase approach to metal ion complexation is fully compatible with split synthesis methodologies employed in combinatorial libraries.

5   The synthesis process was performed using commercial automated synthesizers. Multiple manual synthesizers (such as those commercially available from SynPep Corporation, Dublin, CA) allow parallel synthesis of ten peptides simultaneously.

        Quality control protocols were employed as required, and include HPLC, mass spectral analysis, and amino acid analysis on each individual pool of 25 compounds. The presence of each f

10   pool constituent is established by molecular ion mass spectral analysis. Negative ion mode electron spray (ES) and matrix-assisted laser desorption (MALDI) techniques were employed. Using mass spectral analysis, three different measures were made: (a) the presence of up to 25 individual compounds by molecular ion peak measurement (assuming different masses for each compound), (b) confirmation that the molecular ion peaks show complexation to a rhenium metal ion, and (c) absence

15   of peaks with molecular masses corresponding to peptides uncomplexed with metal ion. Rhenium is a mixture of two isotopes that differ in mass by 2 units (186 and 188) with a relative abundance of these isotopes of 1:2. The molecular ion profile of a metallopeptide appears as two peaks that differ by 2 mass units with integrated area ratios of 1:2. Rhenium thus acts as an internal mass spectral reference for these metallopeptides. A spectral analysis of one such pool of 25 compounds

20   synthesized by the methods of this claim is shown at **Fig. 9**. Five sets of two metallopeptides in this pool have similar masses due to the presence of the same amino acids assembled in different sequences. The relative intensities of the peaks is due to differential ionization of individual compounds in the pool and does not reflect the relative amounts in the mixture. Each pair of peaks with mass unit differences of 2 and relative ratios of 1:2 are due to the relative abundance of two stable

25   isotopes of rhenium (Re-185 and Re-187). The spectral analysis did not reveal any free uncomplexed linear peptides, which would be approximately 197 to 199 mass units less than the corresponding metallopeptide, due to the absence of the rhenium-oxo core.

        Amino acid analysis of each pool of 25 metallopeptides was also employed, and was used to determine the relative equimolar ratio of each of 25 compounds in a pool. The synthetic protocols of

30   split synthesis were designed to assure equimolar amounts of pool constituents.

        <u>Example 3</u>   SCREENING OF MELANOCORTIN RECEPTOR-SPECIFIC LIBRARY

        Metallopeptide library pools are screened for MC4-R receptor and MC1-R receptor binding activity in high throughput screening assays. The MC receptor-binding assay uses membrane preparations from B16-F1 or B16-F10 melanoma cells as the source of MC receptor. C ll membran s

35   prepared from MC4-R-expressing 293 cells and negative control, untransfected 293 cells, ar substituted for B16-F1 or B16-F10 melanoma cell membranes in MC4-R specific binding assays. The MC r ceptor-binding assays use the Millipore Multi-Screen System and ar p rform d in 96-well Millipore filter plates (Durapore, 0.45 mm porosity) pre-blocked with 0.5% bovine serum albumin in

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phosphate buffered saline. Cell membran pr parations (12.5 µg/w ll) are incubated
with 0.4 nM ^{125}I-NDP-MSH in HEPES Buffer containing 0.2% bovine s rum albumin. Non-specific
binding is determined by addition of 10^{-6}M α-MSH or 10^{-7}M NDP-MSH. Metallopeptides to be tested
are added to reaction wells at a final concentration of 1 mM. After incubation for 90 minutes at room

5   temperature, the binding reaction is rapidly terminated by filtration to capture the membranes. Filters
are washed 3 times with ice-cold PBS and air-dried. Individual filters are then punched from the plat s
and distributed into gamma counter tubes. Radioactivity associated with the membranes is determined
in a Packard Cobra gamma counter. Specific binding is determined as the radioactivity in wells
containing ^{125}I-NDP-MSH alone minus the radioactivity in wells containing 10^{-6}M α-MSH. Test

10   compounds are screened in duplicate wells and are considered to be active where 1 µM
concentrations inhibit >50% of the specific binding. Standard curves of unlabeled NDP-MSH will be
included on each plate as an internal assay control.

A commercially available cAMP kit (R&D Systems, DE0350, low pH) is employed to evaluate
agonist potential of metallopeptides that bind to MC4-R. 293 cells stably transfected with hMC-4

15   receptor, or B16-F1 melanoma cells, are grown to confluence in 96-well dishes. Cells are washed and
fresh RPMI containing 0.2 mM isobutylmethylxanthine (cAMP phosphodiesterase) and varying
concentrations of metallopeptides, or α-MSH as a positive control, are added, and the cells are
incubated for 1 hour at 37°C. Medium is aspirated, and cell layers extracted with 150 µl of 0.1 M HCl.
Total cAMP accumulation in 100 µl of cell extract is quantitated in 96-well plates by competitive

20   immunoassay with the cAMP kit, using an acetylation modification. EC_{50} values for test compounds
will be calculated based on cAMP accumulation in cells treated with 10^{-6}M α-MSH. The capabilities of
both of these cell types to accumulate cAMP in the presence of α-MSH and MSH analog peptides ar
documented in the scientific literature; see, for example, Ollman MM et al: *Science* 278:135-138, 1997.

Example 4   DECONVOLUTION OF MELANOCORTIN RECEPTOR-SPECIFIC LIBRARY

25   Deconvolution of a positive pool is done by iterative re-synthesis and screening deconvolution
approaches. The individual 25 constituents are synthesized separately, or alternatively in 5 smaller
pools of 5 compounds each, with each pool screened in receptor binding assays. The latter approach
is preferred where there is a high hit frequency in the preliminary screen. The compounds in pools
with the best results (closest to receptor affinity in the nanomolar range and MC4-R to MC1-R

30   selectivity of at least 100) are individually synthesized and screened.

Example 5   ALTERNATIVE METHOD OF DECONVOLUTION OF MELANOCORTIN
             RECEPTOR-SPECIFIC LIBRARY

In this example, an alternative method of mass spectral deconvolution of metallopeptide
libraries is employed. The method is based on the internal signatur of rhenium-complexed p ptid s

35   (two isotopic p aks in 1:2 ratios differing by 2 mass units), which generally p rmits metallopeptid
identification even in mix d solutions. A positiv pool is incubat d with receptor-bearing cells, th
excess unbound compounds washed away under controlled conditions, and th c lls treat d with a
solvent to disrupt metallopeptide binding and extract th m tallopeptide in the solvent. Mass spectral

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analysis of the solvent rev als the metallopeptide r m tallopeptides which are bound to th receptor-b aring cells, and through comparison to the quality control data it is possible to ascertain the sp cific m tallopeptide or metallop ptides which are bound. This process provides high thr ughput of metallopeptide library screening.

5   Example 6   SINGLE POT SYNTHESIS OF A LIBRARY OF FOUR METALLOPEPTIDES OF
                     THE GENERAL STRUCTURE Ac-His-Xaa-Cys-Trp-NH$_2$

A synthesis procedure similar to that described in **Example 1** was used in making this library. A NovaSyn TGR resin for making peptide amides (substitution 0.2 mM/gm) was used. Fmoc synthetic strategy was employed using the following protected amino acids: Fmoc-Trp(Boc), Fmoc-Cys(S^{t}Bu),

10   Fmoc-Xxx, and Fmoc-His(Trityl). The Xaa amino acids were Trp, HomoPhe, 2'-Naphthylalanine, and Phenylglycine. The peptide resin Cys(S^{t}Bu)-Trp-NH$_2$ was split into four equal pools and one of the Xaa amino acids was coupled to one individual pool. After completion of the coupling reaction, the four resin pools were mixed again. The synthesis proceeded with the coupling of His followed by acetylation of the N-terminus. After the complete assembly of the peptide chain Ac-His(Trt)-Xaa-

15   Cys(S^{t}Bu)-Trp(Boc)-NH$_2$, the S^{t}Bu group was removed by treatment with DMF/tributylphosphine and rhenium-oxo metal ion was complexed as generally described above. The fully protected metallopeptide was deblocked and liberated from the solid support by treatment with a cleavage cocktail (95:5 mixture of trifluoroacetic acid - triisopropylsilane) for three hours. The metallopeptide library was recovered by precipitation using cold ether. The resulting pellet was washed twice and 0.5

20   ml of 95% acetic acid was added. After one-half hour 5 ml of water was added and the solution was freeze-dried yielding the desired library in solid form. Mass spectrometric analysis of the library pool confirmed the correct masses for all four members of the library:

TABLE 3

Compound	Structure	Calculated Mass	Mass (M+1) found
1	Ac-His-Phg-Cys-Trp-NH$_2$	815.7 and 817.6	815.2 and 816.7
2	Ac-His-Trp-Cys-Trp-NH$_2$	868.8 and 870.7	868.0 and 870.1
3	Ac-His-HPhe-Cys-Trp-NH$_2$	843.8 and 845.7	842.8 and 845.2
4	Ac-His-2'Nal-Cys-Trp-NH$_2$	880.0 and 881.9	879.1 and 880.9

25   As noted in **TABLE 3**, two molecular ion peaks differing in mass units of 2 were calculated and observed for each structure; this difference is presumptively due to the presence of two natural isotopes of rhenium, Re-185 and Re-187, in the compl xation step. In addition, the ar a under the obs rved p aks in the spectrometric analysis showed that for each structur the area was in a 1:2 ratio, which is identical to and presumptively related to th relative abundance of Re-185 and Re-187

30   isotop s. These results confirmed the complexation of rhenium to the peptid s. Th spectral analysis

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is shown at **Fig. 10**.

These results were also confirmed by HPLC analysis of this library of four compounds, which results were compared to HPLC analysis of each of the four individual members performed under identical HPLC conditions. As is evident from **Fig. 11**, each of the four member components are present in the library mixture. In the HPLC profile each of the metallopeptide has resolved into two isomers due to alternate orientations (syn and anti) of the rhenium oxo core. The HPLC analysis also revealed the lack of uncomplexed linear peptides in the preparation. All four compounds used for this comparison were individually prepared using methods identical to that described above for synthesis of the library. The HPLC profiles are shown as **Figs. 11A to 11E**.

Example 7   SYNTHESIS OF MC1-R SPECIFIC METALLOPEPTIDES FOR USE AS CHEMOPREVENTION AGENT

High potency metallopeptides are also provided with N-terminal modifications. Systematic N-terminal modifications are made based upon the limited data available in the literature related to receptor-binding affinities of peptide analogs for various MC receptor types. In general, these studies indicate that the His6 residue may be a critical factor in determining receptor selectivity for MC1-R (peripheral) versus MC4-R (brain). Three-dimensional molecular models of the human melanocortin receptor have been developed based upon the electron cryo-microscopic structure of bacteriorhodopsin and the electron density footprint of bovine rhodopsin. By modeling known potent agonists into the proposed binding sites, specific ligand-receptor interactions have been identified. By this means, researchers have tentatively identified melanotropin side-chain pharmacophores, D-Ph [7] and Trp[9], and have proposed that these interact with a hydrophobic network of receptor aromatic residues in transmembrane regions 4, 5, 6, and 7. The findings of these results are utilized in selecting N-terminal modifications for the metallopeptide core. Groups with hydrophobic and/or hydrogen bonding potential are investigated in a systematic and stereospecific manner, with contemporaneous assaying of potency and MC1-R specificity of the resulting metallopeptides. The influence of the His residue on bioactivity is also investigated. Two series of metallopeptides are synthesized:

Rheniumoxo - [R-His-D-Phe-Arg-Cys-Trp-NH$_2$] and
Rheniumoxo - [R-D-Phe-Arg-Cys-Trp-NH$_2$]

Where R is a pair consisting of a hydrophobic side chain and hydrophilc side chain with hydrogen bonding potential which is selected from the following groups:

Ser;   HSer;   HO-(CH$_2$)n-CO-;

H$_2$N-(CH$_2$)$_n$-CO-;   HOOC-(CH$_2$)$_n$-CO-;   CH$_3$-(CH$_2$)n-CO-;

-63-
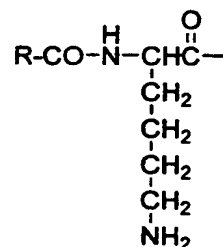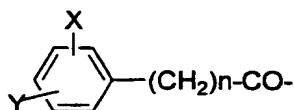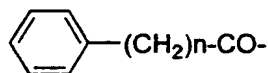
And where n is from 2 to 9 and X and/or Y ar selected from H, OH, Cl, Br, I, NH$_2$, OCH$_3$, NO$_2$ and similar groups.

<u>Example 8</u>   SKIN DARKENING IN ANOLIS CAROLINENSIS

PT-1145 complexed with rhenium (0.65 mg taken in a 50 mL vehicle) was injected intraperitoneally in a lizard (Anolis carolinensis) that was pre-conditioned for a skin darkening experiment. The pre-conditioning involved leaving the lizards in a well-lit white background for 24 hours. Within 10-15 minutes after injection, the skin coat color turned from bright green to dark brown to black. The skin coat color remained dark during the five-hour observation period. Lizards injected with the vehicle alone (PBS buffer containing 1 % each of DMF and beta-cyclodextran) did not show any change in their skin color during the 5-hour observation period.

Each of the foregoing is merely illustrative, and other equivalent embodiments are possible and contemplated.

Although this invention has been described with reference to these preferred embodiments, other embodiments can achieve the same results. Variations and modifications of the present invention will be obvious to those skilled in the art and it is intended to cover in the appended claims all such modifications and equivalents. The entire disclosures of all applications, patents, and publications cited above are hereby incorporated by reference.

The preceding examples can be repeated with similar success by substituting the generically or specifically described reactants and/or operating conditions of this invention for those used in the preceding examples.